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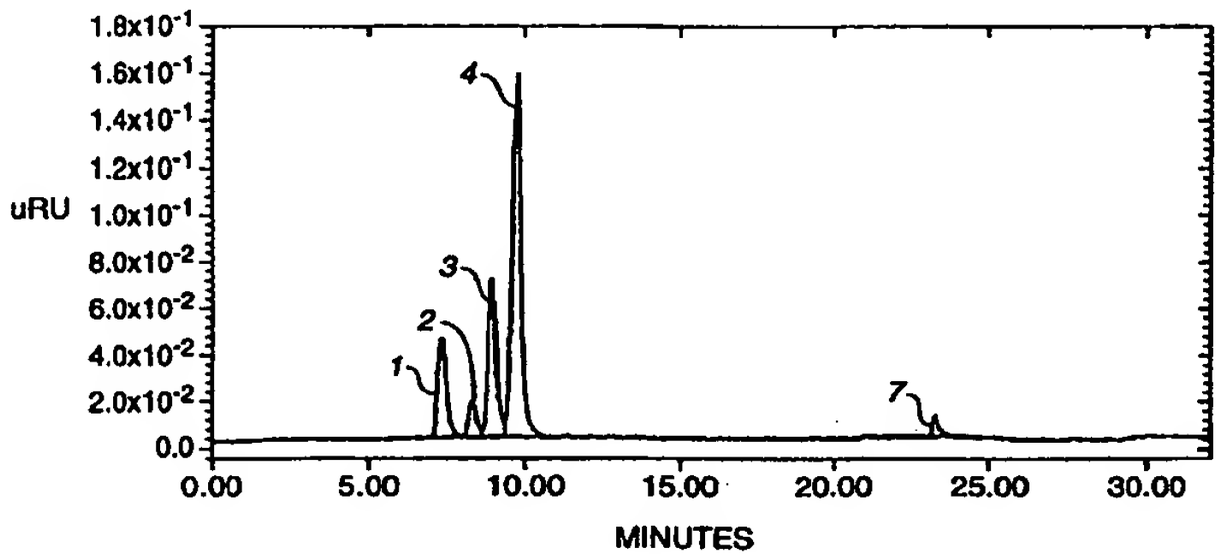
(54) Title: BIFUNCTIONAL RESIN COMPOSITION FOR THE SIMULTANEOUS SEPARATION OF CARBOHYDRATES AND ORGANIC ACIDS IN LIQUID CHROMATOGRAPHY

(57) Abstract

An improved bifunctional chromatography resin composition which allows for the separation of both carbohydrates and organic acids during a single liquid chromatography run. The resin compositions comprise synthetic resin support particles to which are attached (1) ligand exchange functional groups which comprise a metal cation useful for the separation of carbohydrates in mixtures thereof, and (2) anion exchange functional groups which are capable of separating organic acids from mixtures thereof. The ligand exchange and anion exchange functional groups function independently in the resin composition, thereby allowing the separation of both carbohydrates and organic acids in a single liquid chromatography run.



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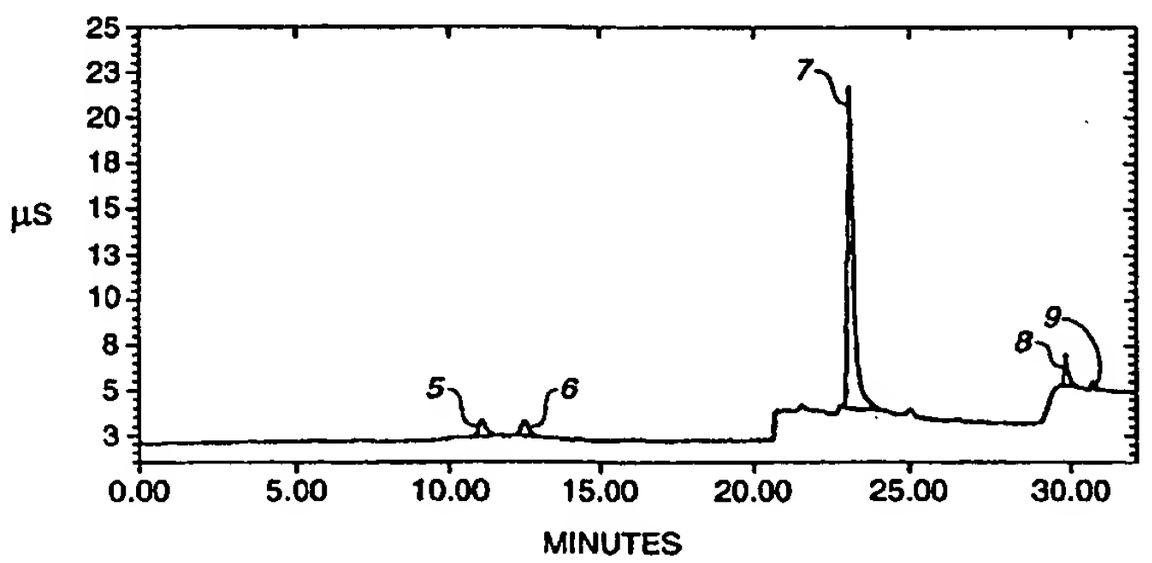

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BIFUNCTIONAL RESIN COMPOSITION FOR THE SIMULTANEOUS SEPARATION OF CARBOHYDRATES AND ORGANIC ACIDS IN LIQUID CHROMATOGRAPHY

FIELD OF THE INVENTION

The present invention concerns novel liquid chromatography resin compositions and methods useful for the simultaneous separation of carbohydrates and organic acids in liquid chromatography.

BACKGROUND OF THE INVENTION

The present invention relates to novel resin compositions which are useful for performing improved liquid chromatography. More particularly, the present invention relates to improved bifunctional chromatographic resin compositions and methods for performing liquid chromatography using those resin compositions where the resin is capable of simultaneously separating carbohydrates and organic acids in a single chromatographic run. Such bifunctional resin compositions find use in a variety of different applications including, for example, in the analysis of carbohydrates and organic acids in consumable food and beverage products.

There are several commonly employed methods for the analysis and separation of carbohydrates in liquid chromatography. For example, carbohydrates may be efficiently separated by a reverse phase mechanism

which employs a standard high pressure liquid chromatography (HPLC) column, often consisting of a C-18 silica-based stationary phase (El Rassi, *J. Chromatography A* 720:93-118 (1996)). When such a stationary phase is employed, carbohydrate separation is typically accomplished using a mostly aqueous eluent containing a small amount of solvent to control elution of the different carbohydrate species.

Carbohydrates may also be effectively separated using normal phase chromatography on an amino group-bonded stationary phase (Churms, *J. Chromatography A* 720:75-91 (1996)). This type of separation is characterized by the formation of strong interactions between hydroxyl groups of the carbohydrate species and amino groups present on the stationary phase. The amino groups employed are typically bonded to a standard silica-based solid phase substrate wherein the eluent consists primarily of a high solvent matrix (for example, 80% to 90% acetonitrile).

A third method which is often employed to separate carbohydrates in liquid chromatography is high pH anion exchange chromatography on a pellicular anion exchange resin (Lee, *J. Chromatography A* 720:137-149 (1996)). In this case, the stationary phase is a strongly basic polymeric anion exchange material which is used in conjunction with hydroxide-based eluent systems. In high pH anion exchange chromatography, the hydroxide eluent acts to convert carbohydrates to their anionic forms where different carbohydrate species may then be separated based upon their different pKas.

A fourth method useful for separating carbohydrates in liquid chromatography involves a ligand-exchange mechanism on a metal-loaded, fully sulfonated cation exchange resin (Stefansson and Westerlund, *J. Chromatography A* 720:127-136 (1996)). For such separations, the cation exchange resin is typically loaded with a metal such as calcium or potassium, wherein the eluent employed is typically water. Effective carbohydrate separation on such a resin, however, requires that the chromatography be performed at temperatures in the range of about 60°C to 80°C. Such elevated temperatures are required because the

carbohydrate species exist in several different anomeric forms which have different column retention times, and a low rate of interconversion between the different anomeric forms results in a less than effective separation. By operating the chromatography at elevated temperatures, however, the interconversion rate of the anomeric forms of the carbohydrates increases, thereby significantly enhancing the ability to separate the different species.

Like the analysis of carbohydrates, there are also several commonly applied methods for the analysis and separation of organic acids. For example, underivatized organic acids may be chromatographically separated using reverse phase HPLC, wherein the stationary phase is typically a C-18 bonded silica-based substrate which employs an eluent of water containing either methanol or acetonitrile (Manning and Maskarinec, *J. Liquid Chromatography* 6(4):705-714 (1983)). However, while such chromatographic systems are capable of effectively separating some organic acids, they provide relatively poor resolving power for divalent carboxylic acids and even poorer resolving power for high valency carboxylic acids such as citrate and isocitrate.

A second method used for the chromatographic separation of organic acids is "ion exclusion" which employs as a stationary phase a fully sulfonated cation exchange resin in the hydrogen form (Fritz, *J. Chromatography* 546:111-118 (1991) and Widiastuti and Haddad, *J. Chromatography* 602:43-50 (1992)). The "ion exclusion" mechanism is based upon the fact that a given organic acid is in equilibrium with its neutral form to an extent that varies both with the eluent pH and the pKa of the organic acid. The neutral form of the acid is free to partition into the highly acidic sulfonic acid stationary phase while the ionic form of the acid is excluded from the stationary phase due to electrostatic repulsion. As a result, more highly ionized analytes are retarded by the column to a lesser extent than are less ionized analytes. Such a mechanism, however, is limited in that it is capable of only separating up to about 12 different organic acids in a single chromatographic run.

Finally, organic acids may also be separated using anion exchange chromatography which typically employs any of a number of different anion exchange resins and a carbonate- or hydroxide-based eluent system.

The above examples evidence that various chromatographic systems have been developed for the separation and analysis of carbohydrates or organic acids. Attempts to develop bifunctional chromatographic systems that are capable of separating both carbohydrates and organic acids in a single run, however, have been for the most part unsuccessful. For example, McFeeters, *J. Agricultural Food Chemistry* 41:1439-1443 (1993) and Lopez and Gomez, *J. Chromatographic Science* 34:254-257 (1996) describe the simultaneous separation of carbohydrates and organic acids on a sulfonated cation exchange resin in the hydrogen form. However, such a separation mechanism is wholly inadequate for the chromatographic separation of real food samples because the hydrogen form of a cation exchange resin possesses a relatively poor ability to separate carbohydrates. As a consequence, the technique is of little or no practical value for the analysis of real samples containing both carbohydrates and organic acids.

There is, therefore, a need for novel bifunctional resin compositions which are capable of efficiently separating both carbohydrates and organic acids in a single chromatographic run. There is also a need for bifunctional resin compositions which are capable of separating both carbohydrates and organic acids at room temperature, thereby eliminating the need for elevated temperatures as required by certain known separation mechanisms.

SUMMARY OF THE INVENTION

In accordance with the present invention, a novel bifunctional resin composition has been formed which provides the ability to separate both carbohydrates and organic acids in a single liquid chromatography run, wherein the resin finds particular use in the analysis of carbohydrates and organic acids in food samples. More particularly, the present invention is based at least in part on the finding that by employing a bifunctional

stationary phase having (1) positively-charged metal ion containing ligand exchange functional groups which are capable of interacting with the hydroxyl groups on carbohydrates, thereby serving as a means for separating different carbohydrates in a mixture thereof, and (2) standard anion exchange sites which are capable of interacting with organic acids, thereby providing a means for separating different organic acids in a mixture thereof, one may efficiently and effectively separate both carbohydrates and organic acids in a single chromatographic run. The present invention, therefore, provides a novel bifunctional stationary phase and methods of use thereof which provide unique separation characteristics and which find use in a variety of different applications.

Therefore, one aspect of the present invention is directed to a bifunctional chromatography resin composition capable of simultaneously separating both carbohydrates and organic acids in a sample stream comprising the carbohydrates and organic acids which is flowing through the resin composition, wherein the bifunctional resin composition comprises:

(a) synthetic resin support particles;

(b) ligand exchange functional groups attached to the synthetic resin support particles, wherein the ligand exchange functional groups comprise one or more metal cations, and

(c) anion exchange functional groups attached to the synthetic resin support particles. The metal cation-containing ligand exchange sites are capable of interacting with at least one of the carbohydrates in the sample stream which is flowing through the resin composition, thereby delaying the elution of that carbohydrate from the resin compositon. Additionally, the anion exchange functional groups are capable of interacting with at least one organic acid present in the sample stream, thereby delaying the elution of that organic acid from the resin composition. The metal cations of the ligand exchange functional groups may comprise, for example, potassium, calcium, cesium, sodium, strontium and/or barium cations, wherein the ligand exchange functional groups may be attached to internal surfaces defined by pores in the

synthetic resin support particles. The anion exchange functional groups may comprise quaternary ammonium groups and may be attached to the surface of the synthetic resin support particles by covalent bonding or by electrostatic attachment of a latex polymer on the surface of the support particles.

Another aspect of the present invention is directed to a chromatography bed which comprises the above described bifunctional resin composition, wherein the bed may optionally be placed in fluid communication with a detector for carbohydrates and organic acids that are eluting from the bed. Such detectors include, for example, refractive index detectors, conductivity detectors and/or amperometric detectors, which may be used in series and/or with a suppressor.

A further aspect of the present invention is directed to methods for making the above described bifunctional resin composition, wherein those methods comprise attaching metal cations to negatively charged sites on surfaces within synthetic resin support particles to provide ligand exchange sites on those surfaces, and

attaching anion exchange functional groups to the surface of the synthetic resin support particles to form the bifunctional resin composition.

Yet another aspect of the present invention is directed to methods for separating a first carbohydrate or organic acid from a second carbohydrate or organic acid in a sample stream comprising the first and second carbohydrates and/or said first and second organic acids wherein the first and second carbohydrates or organic acids are different, the method comprising flowing the sample stream through a chromatography bed comprising the above described bifunctional resin composition in the presence of a hydroxide ion containing eluent, whereby the first carbohydrate or organic acid interacts with functional groups in the resin composition and thereby elutes off the chromatography bed later than the second carbohydrate or organic acid.

Other aspects of the present invention will become evident upon a reading of the present specification.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Process For Preparing Synthetic Resin Support Particles Possessing Metal Cation-Containing Ligand Exchange Functional Groups. Presented is a diagram showing an embodiment of a method for the preparation of a synthetic resin particle having metal cation-containing ligand exchange functional groups attached thereto. Specifically, a sulfonated resin particle having a hydronium counterion is filtered and washed with a metal cation containing solution such as potassium hydroxide or potassium chloride, wherein the hydronium counterion is replaced by the metal cation to form the ligand exchange site.

Fig. 2. Carbohydrate Ligand Exchange Process. Presented is a schematic diagram showing the mechanism by which hydroxyl groups on carbohydrate molecules exchange at metal cation-containing ligand exchange sites.

Fig. 3. Organic Acid Anion Exchange Process. Presented is a schematic diagram showing the mechanism by which an organic acid exchanges with a mobile phase anion on a quaternary ammonium anion exchange functional group.

Fig. 4A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of Apple Juice on a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of apple juice on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of apple juice on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-sorbitol, 3-glucose, 4-fructose, 5-quinic acid, 6-lactic acid, 7-malic acid, 8-phosphate and 9-citric acid.

Fig. 5A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of Orange Juic on a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of orange juice on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of orange juice on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-glucose, 3-fructose, 4-quinic acid, 5-acetate, 6-chloride, 7-succinic acid, 8-tartaric acid, 9-oxalic acid, 10-phosphate and 11-citric acid.

Fig. 6A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of Cranberry Juice on a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of cranberry juice on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of cranberry juice on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-maltose, 2-glucose, 3-fructose, 4-quinic acid, 5-lactic acid, 6-unknown, 7-chloride, 8-succinic acid, 9-oxalic acid and 1-citric acid.

Fig. 7A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of Grapefruit Juice on a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of grapefruit juice on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of grapefruit juice on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-glucose, 3-fructose, 4-chloride, 5-malic acid, 6-sulfate, 7-oxalic acid, 8-phosphate and 9-citric acid.

Fig. 8A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of Swiss Mocha on a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of Swiss Mocha on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of Swiss Mocha on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-unknown, 3-glucose, 4-unknown, 5-quinic acid, 6-unknown, 7-unknown, 8-lactic acid, 9-unknown, 10-chloride, 11-succinic acid, 12-sulfate, 13-nitrate, 14-oxalic acid, 15-phosphate and 16-citric acid.

Fig. 9A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of Grape Juice on a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of grape juice on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of grape juice on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-fructose, 3-quinic acid, 4-galacturonic acid, 5-chloride, 6-malic acid, 7-tartaric acid, 8-sulfate, 9-phosphate, 10-citric acid and 11-unknown.

Fig. 10A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of Red Grape Must on a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of red grape must on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of red grape must on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-fructose, 3-fluoride, 4-lactic acid, 5-galacturonic acid, 6-chloride, 7-malic acid, 8-tartaric acid, 9-sulfate, 10-phosphate and 11-citric acid.

Fig. 11A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of White Grape Must n a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of white grape must on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of white grape must on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-fructose, 3-unknown, 4-fluoride, 5-lactic acid, 6-galacturonic acid, 7-chloride, 8-malic acid, 9-tartaric acid, 10-sulfate, 11-phosphate and 12-citric acid.

Fig. 12A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of Merlot Wine on a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of merlot wine on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of merlot wine on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-ethanol, 3-lactic acid, 4-acetic acid, 5-galacturonic acid, 6-chloride, 7-malic acid, 8-tartaric acid, 9-unknown, 10-sulfate and 11-phosphate.

Fig. 13A and B. Refractive Index and Conductivity Profile Obtained From an Analysis of a Synthetic Mixture of Carbohydrates and Organic Acids on a Potassium-Containing Bifunctional Resin Composition. A. Presented is the refractive index elution profile obtained from an analysis of a synthetic mixture of carbohydrates and organic acids on a potassium-containing bifunctional resin composition of the present invention. **B.** Presented is the conductivity elution profile obtained from an analysis of a synthetic mixture of carbohydrates and organic acids on a potassium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-maltose, 3-glucose, 4-fructose, 5-ethanol, 6-

quinic acid, 7-lactic acid, 8-acetic acid, 9-formic acid, 10-pyruvic acid, 11-succinic acid, 12-tartaric acid, 13-oxalic acid and 14-citric acid.

Fig. 14. Refractive Index Profile Obtained From an Analysis of a Synthetic Mixture of Carbohydrates and Organic Acids on a Cesium-Containing Bifunctional Resin Composition. Presented is the refractive index elution profile obtained from an analysis of a synthetic mixture of carbohydrates and organic acids on a cesium-containing bifunctional resin composition of the present invention. Peak numbers are as follows: 1-sucrose, 2-maltose, 3-glucose, 4-fructose, 5-quinic acid, 6-lactic acid, 7-acetic acid, 8-formic acid, 9-pyruvic acid, 10-succinic acid, 11-malic acid, 12-tartaric acid, 13-oxalic acid and 14-fumaric acid.

DETAILED DESCRIPTION OF THE INVENTION

A. Bifunctional liquid chromatography resin compositions

The bifunctional liquid chromatography resin compositions of the present invention comprise synthetic resin support particles where attached to those particles are (1) ligand exchange functional groups which are capable of interacting with at least one carbohydrate present in a sample stream flowing through the resin composition, and (2) anion exchange functional groups which are capable of interacting with at least one organic acid present in the sample stream. Although the resin particles comprise both ligand exchange and anion exchange functional groups, those groups function independently of one another and may be made part of the chromatographic resin compositions in an independent manner. The resin compositions, therefore, are "bifunctional" in that they possess both (1) ligand exchange functional groups useful for the separation of carbohydrates, and (2) anion exchange functional groups useful for the separation of organic acids, both of which function separately and independently of the other.

The synthetic resin support particles of the presently described chromatographic compositions may be organic or inorganic in nature and may be formed from any suitable insoluble material which will support the

attachment of the ligand exchange and anion exchange functional groups described below. For example, synthetic polymer ion-exchange resins such as poly(phenol-formaldehyde), polyacrylic, or polymethacrylic acid or nitrile, amine-epichlorohydrin copolymers, graft polymers of styrene on polyethylene or polypropylene, poly(2-chloromethyl-1,3-butadiene), poly(vinylaromatic) resins such as those derived from styrene, alpha-methylstyrene, chlorostyrene, chloromethylstyrene, vinyltoluene, vinylnaphthalene or vinylpyridine, corresponding esters of methacrylic acid, styrene, vinyltoluene, vinylnaphthalene, and similar unsaturated monomers, monovinylidene monomers including the monovinylidine ring-containing nitrogen heterocyclic compounds, copolymers of the above monomers, silica and C-18-derivatized silica are all suitable.

The size range for synthetic resin support particles which find use in the present invention is typically from 2-100 microns, preferably from 5-20 microns, although significantly smaller or larger support particles may also find use. The particles may be either macroporous or microporous depending upon the particular application desired, however, microporous particles are preferred so as to acheive maximum capacity when fully sulfonated. Microporous particles are those having average pore sizes of about 50 angstroms or less whereas macroporous particles have average pore sizes of greater than about 50 angstroms.

The synthetic resin support particles of the present invention can be formed, for example, by well known suspension polymerization techniques which involve suspending droplets of monomer in an aqueous medium in which it is insoluble. Under suitable conditions, the monomer will polymerize. This can be accomplished by mixing the monomer with additives in a suspension medium. When this medium is agitated, the monomer disperses into droplets and agitation continues until polymerization is complete. Preferably, the synthetic resins used are of the microporous type which are well known in the art, particularly including styrene-divinylbenzene copolymer. The copolymer can be prepared, for example, according to the method of Ikada et al., *Journal of Polymer Science* 12:1829-1839 (1974) or as described in U.S. Patent No.

4,382,124 to Meitzner et al. Other techniques for the synthesis of synthetic resin support particles are well known in the art and can be found in U.S. Patent Nos. 3,915,642, 3,918,906, 3,920,398, 3925,019 and the monograph "Dowex: Ion Exchange" 3rd. edition, (1964) published by the Dow Chemical Company, Midland, Michigan.

Preferably, the synthetic resin support particles comprise beads of cross-linked polymer or copolymer, such as styrene-divinylbenzene copolymer which copolymerize in the presence of catalyst such as benzoyl peroxide, containing between about 0% to about 100% divinylbenzene monomer by weight. More preferably, the styrene-divinylbenzene copolymer contains between about 4% to about 16% divinylbenzene monomer by weight. The styrene-divinylbenzene-based particles may optionally comprise a third monomer such as an acrylate- or methacrylate-based monomer which, subsequent to sulfonation (see below), provides carboxylic groups attached to the synthetic support as well as sulfonate groups. A detailed review of the preparation, structure and morphology of styrene-based polymers is provided by Guyot and Bartholin, *Prog. Polym. Sci.* 8:277-332 (1982).

The neutral synthetic resin support particles may then be sulfonated so as to provide $-SO_3^-$ substituted sites therein at which the ligand exchange functional groups may be created or the anion exchange functional groups may be attached. After sulfonation, the synthetic resin support particles constitute a conventional ion exclusion type of resin having reactive sulfonate groups where the counterion is hydronium ion. Methods for sulfonation of neutral synthetic resin support particles are well known and routinely used in the art. While sulfonation of the resin support particles is preferred, carboxylation and/or phosphonation may also be employed to provide reactive sites at which ligand exchange sites may be created or anion exchange groups may be attached (see, e.g., Kolla et al., *Chromatographia* 23:465 (1987)).

The sulfonated synthetic resin support particles of the bifunctional resin compositions described herein contain "ligand exchange functional groups" or "ligand exchange sites". Ligand exchange functional groups

comprise a metal cation which is capable of interacting with the hydroxyl groups on carbohydrates, thereby retaining those carbohydrate molecules for a longer time relative to other carbohydrate molecules with which the metal cation interacts less efficiently. Metal cation-containing ligand exchange functional groups are known in the art and their structure and mechanism of action is described by Stefansson and Westerlund (1996), *supra*.

Ligand exchange functional groups which find use herein may be added to the synthetic support particles by filtering the above described sulfonated support particles with a base or salt solution wherein the cation of the base or salt solution is a positively charged metal ion. Filtering of the sulfonated synthetic resin support particles with a base or salt solution causes replacement of the hydronium counterion with a metal cation which will serve as the ligand exchanger, wherein the metal cation can be potassium, calcium, sodium, cesium, strontium, barium, or any other like positively charged metal, preferably potassium. The process of creating a potassium-containing ligand exchange site is schematically illustrated in Fig. 1. The ligand exchange process between the metal cation-containing ligand exchange functional groups and carbohydrate molecules is schematically illustrated in Fig. 2 which shows that the metal cation is capable of interacting with hydroxyl groups present on carbohydrates, thereby retarding the elution of the carbohydrate from the resin composition.

While the metal cation-containing ligand exchange functional groups may exist on any surface of the synthetic resin support particles that is exposed to the eluent, it is preferable to attach all or a majority of them to internal surfaces defined by pores in the synthetic resin support particles so as to spatially separate the ligand exchange sites (used for carbohydrate separation) from the anion exchange functional groups (used for organic acid separation) which will preferably be attached to the outer spherical surface of the resin support particles. By "internal surface defined by a pore" in a synthetic resin support particle is meant a surface of a pore in the resin support particle which is exposed to the external

aqueous environment surrounding the resin support particles but which is not on the immediate outer spherical surface of the support particles.

The amount of ligand exchange capacity exhibited by the resin composition is a direct function of the degree to which the neutral synthetic resin support particles were originally sulfonated, carboxylated, phosphonated, etc. In other words, since sulfonated, carboxylated, phosphonated, etc. sites on the surface of the synthetic support particle are exploited to create the metal cation-containing ligand exchange sites (as schematically illustrated in Fig. 1), one may routinely control the ligand exchange capacity of the resin composition by controlling the degree to which the originally neutral synthetic resin support particles are sulfonated, carboxylated, or phosphonated. Methods for controlling these reactions are well known and routinely used in the art.

The ligand exchange sites of the herein described resin compositions function separately and independently of the anion exchange functional groups which are also attached to the resin support particles. The ligand exchange sites are capable of interacting with one or more carbohydrate molecules present in a sample stream flowing through the resin composition, thereby delaying the elution of the carbohydrate molecules with which they interact as compared to those carbohydrate molecules with which they do not interact or with which they interact with a lesser affinity. The ligand exchange functional groups are capable of interacting with any hydroxyl group-containing carbohydrate molecule (see Fig. 2) including, for example, sucrose, maltose, glucose, fructose, galactose, maltotriose, stachyose, raffinose, arabinose, ribose, mannitol, sorbitol, xylitol, and the like.

Also attached to the synthetic resin support particles of the bifunctional liquid chromatography resin of the present invention are anion exchange sites. "Anion exchange functional groups" or "anion exchange sites" are functionalities which are capable of interacting with at least one organic acid present in a sample stream which is flowing through the bifunctional resin, wherein the interaction serves to retard the elution of that organic acid from the resin. Typical anion exchange functional

groups are well known in the art and include, for example, terminal quaternary ammonium groups having the formula $-XNR_3R_4R_5$ where "N" is a nitrogen atom, "X" is the group within the polymer to which the nitrogen atom is attached and R_3, R_4 and R_5 are, for example, each independently straight-chain or branched alkyl from 1 to about 12 carbon atoms or straight-chain or branched hydroxyalkyl from 1 to about 12 carbon atoms. Preferably, R_3, R_4 and R_5 are each independently methyl, ethyl, propyl, isopropyl, butyl, isobutyl or hydroxyalkyl, more preferably, hydroxyalkyl. The terminally-located anion exchange functional group may optionally be attached to the synthetic resin support particle through a linker group of variable length and size which is generally at least about 3 atoms in length, more generally more than about 4 atoms in length, preferably from about 4 to 13 atoms in length, which may be branched, unbranched, straight-chain, aromatic, and the like.

Anion exchange functional groups may be created in a variety of different ways. For example, one of the monomer molecules employed in the polymerization of the synthetic resin support particle (see below) may possess a terminal epoxide group or halogen atom which will extend from the surface of the polymerized support particle. In the case where a halogen atom is employed, it is preferably chlorine or bromine. The terminal epoxide group or halogen atom of this "anion exchange precursor molecule" may then be reacted with a tertiary amine using well known techniques to produce a terminal quaternary ammonium anion exchange site which is capable of interacting with organic acids present in an eluent in contact therewith. The reaction creating the terminal quaternary ammonium anion-exchange site may occur prior to grafting the monomer to the surface of the synthetic resin support particle, prior to incorporation of the monomer into a latex polymer or after the monomer is incorporated into a latex polymer (see below).

Preparation of anion exchange monomers that are useful as components for polymerization of a latex polymer which is deposited onto the surface of a synthetic resin support particle or for directly grafting to the surface of a synthetic resin support particle is well within the skill level

of the ordinarily skilled artisan. For example, for the synthesis of acrylate- and methacrylate-based compounds, the first step generally involves the formation of an appropriate alkenyl acrylate followed by epoxidation of that alkenyl acrylate. For general reviews on techniques for the preparation of alkenyl acrylates and the epoxidation thereof, see Korshunov et al., *J. Org. Chem. USSR* 4:990 (1968) and Resowsky et al., *The Chemistry of Heterocyclic Compounds*, Interscience Publishers, Vol. 19, Part I (1964), respectively. Moreover, the synthesis and purification of many of the epoxyalkyl or haloalkyl acrylates described herein have been described previously, such references providing guidance as to the synthesis of other similar compounds (see, for example, Sandler et al., *Polymer Synthesis*, Chapter 10, Academic Press, London (1974), Gladkikh et al., *J. Org. Chem. USSR* 11:1602 (1975), Fort et al., *Tetrahedron* 48:5099 (1992), U.S. Patent No. 2,863,851 and U.S. Patent No. 3,001,975.

While the anion exchange functional groups may exist on any external surface of the synthetic resin support particles, they preferably are attached to the external spherical surface of the synthetic resin support particles so as to be spatially separated from the ligand exchange groups which, as described above, will preferably be attached to surfaces defined by pores in the synthetic resin support particles. In the situation where the majority of ligand exchange groups are attached to surfaces defined by pores in the synthetic support and the majority of anion exchange groups are attached to the external spherical surface of the synthetic support, one is efficiently employing virtually all of the available surface area of the synthetic resin support particles for chromatographic purposes. Such allows for the preparation of small volume chromatography columns having very high separation capacities.

The anion exchange functional groups of the herein described resin compositions function separately and independently of the ligand exchange functional groups described above. The anion exchange functional groups are capable of interacting with one or more organic acid molecules present in a sample stream flowing through the resin

composition, thereby delaying the elution of the organic acid molecules with which they interact as compared to those organic acid molecules with which they do not interact or with which they interact with a lesser affinity. The anion exchange functional groups are capable of interacting with virtually any organic acid molecule (see Fig. 3) including, for example, quinic acid, lactic acid, acetic acid, formic acid, pyruvic acid, succinic acid, tartaric acid, oxalic acid, fumaric acid, citric acid, malic acid, and the like.

The anion exchange functional groups may be "attached" to the surface of the synthetic resin support particles in a variety of different ways. In the preferred case where the synthetic resin support particles have been sulfonated, carboxylated, phosphonated, and the like, the anion exchange groups (or "precursors" thereof) may be incorporated into a positively charged latex which is electrostatically deposited onto the surface of the negatively charged sulfonated synthetic support particles as described in U.S. Patent No. 5,324,752. Specifically, as described in U.S. Patent No. 5,324,752, the preparation of latex polymer involves the polymerization of one or more monomer molecules (having an alkenyl group at one terminus and either the anion exchange group or an anion exchange group precursor which is capable of being converted to an anion exchange group, such as an epoxide group or halogen atom, at the other terminus) with a dialkenyl cross-linking monomer that has an available alkenyl group at each terminus and optionally another monoalkenyl monomer. The amount of the optional monoalkenyl monomer added to the reaction provides a means for diluting or controlling the relative number of anion exchange sites that exist in the final latex polymer product. The above components are polymerized in the aqueous phase to form a suspension of colloidal particles which are commonly called latex which, in turn, are irreversibly attached to the ligand exchange functional group-containing synthetic resin support particles by either (1) electrostatic attachment to the negatively charged particle surface which had been previously sulfonated, carboxylated or phosphonated, or (2) attached to the solid phase via a "dispersant" material that possesses

functional sites that irreversibly attach to both the latex polymer and the solid phase, ther by forming a permanent attachment therebetween (see below). The latex polymerization reaction may be performed by conventional emulsion polymerization techniques, such as by heating and stirring a suspension of monomers in a suitable solvent in the presence of a suitable emulsifying agent. Alternatively, the polymerization may be carried out by a suspension, bulk or solution process followed by grinding the resin to a desired size by mechanical means such as ball mills, rod mills or the like. Latex particle sizes typically range from about 0.0025 microns to 5 microns, although smaller or larger latex particle sizes may also find use herein. Typical ratios of latex particle diameter to synthetic resin support particle diameter range from about 1:5 to 1:1000 although ratios outside this range may also be employed.

The cross-linking and optionally-added monoalkenyl diluent monomers may be formed from many different well-known synthetic reactions. Specifically, cross-linking monomers are molecules possessing alkenyl groups at each terminus wherein the group separating the terminal alkenyl groups may be, for example, aromatic or aliphatic and may possess one or more heteroatoms such as oxygen or sulfur. Optionally-added monomers possess one terminal alkenyl group and may be styrene-, acrylate- or methacrylate-based. Specific dialkenyl cross-linkers which find use in the present invention include, for example, divinylbenzene, diethyleneglycol dimethacrylate and ethylene methacrylate or respective acrylates thereof. Specific optionally-added monoalkenyl monomers include, for example, styrene, methyl methacrylate and 2-ethoxyethyl methacrylate or respective acrylates thereof. Preferably, the relative reactivities of the selected monomer components are similar, thus assuring an approximately even distribution of each of the monomer units in the final latex polymer product.

As described above, the latex polymer may be "deposited" onto the surface of a synthetic resin support particle (and thereby "attached" thereto) by electrostatic attachment to a surface sulfonated, carboxylated or phosphonated particle or via a bridge of "dispersant" material which

irreversibly binds to both the latex polymer and the solid support particles, thereby forming a bridge between (see U.S. Patent No. 5,324,752). Depending upon the solid support particles and the latex polymer, the dispersant may be any material which can inhibit or prevent agglomeration during suspension in the aqueous medium used for polymerization. For example, the dispersant may be selected from any one of the methacrylic acid copolymers, polymaleates, sulfonated polymers, polyvinylpyrrolidone esters, plant-based gums, lignins and cellulose derivatives. In a preferred embodiment, the dispersant material can be formed of polyvinylalcohol, sulfonated lignin, polyvinylpyrrolidine, gum arabic, gelatin, maleic acid-vinylacetate copolymer or styrene-maleic anhydride copolymer. Usually, the dispersant comprises between about 0.1% to about 25% dispersant by weight water.

The irreversible attachment of a dispersant to the synthetic resin support particles can occur by covalent bonding via various mechanisms. One mechanism is by covalent bonding via a free radical polymerization reaction. Free radicals are typically generated in the resin support particle polymer being formed and sustain polymerization of the polymer as well as promote branching, the formation of new chains of bridging and cross-linking. An initiator can be utilized in the polymerization step of the resin support particle which starts and maintains the polymerization reaction. If the initiator concentration is high enough, more free radical sites are generated than can be consumed in the polymerization reaction, and other chemical species that are present, such as dispersant, can react with them. Thus, the dispersant can covalently link to the resin substrate particle polymer. For example, it has been suggested that polyvinylalcohol dispersant can become covalently linked to another polymer if the initiator concentration is high enough. Ikada et al., *Journal of Polymer Science* 12:1829-1839 (1974). While studying the process of particle formation during suspension polymerization, it was observed that polyvinyl chloride can be chemically grafted to the dispersant. Kirk et al., *Encyclopedia of Chemical Technology,* 3rd ed. Vol. 23, pp. 888-890 (1983).

A second method of irreversible attachment of dispersant to the resin support particles can be by permanent physical entanglement. In this mechanism, relatively small polymers, such as sulfonated lignin dispersant or polyvinylalcohol dispersant can become permanently entangled with the resin support particle polymer as the polymerization reaction occurs.

The irreversible attachment of dispersant to the latex polymer produced as described above can occur by covalent bonding, such as described previously herein, or by electrostatic forces. For example, the synthetic resin support particles can be made of styrene-divinylbenzene copolymer and the dispersant can be a mixture of sulfonated lignin and gum arabic dispersant. The sulfonated lignin can irreversibly attach to the synthetic resin support particles either by covalent bonding or by permanent entanglement, thereby providing a negatively-charged surface to which the latex polymer can be agglomerated electrostatically. Specific methods for carrying out the above may be found in U.S. Patent No. 5,324,752. Also, as described above, the surface of the synthetic resin support particle may be sulfonated, carboxylated or phosphonated so as to provide the requisite negative charge for electrostatic attachment of the latex polymer.

In another embodiment, the anion-exchange groups which find use herein may be covalently bonded or grafted to the surface of the synthetic resin support particle by employing the method described in U.S. Patent No. 5,503,933, issued to Afeyan et al. Specifically, in the method described by Afeyan et al., both the anion exchange group (or precursor thereof) being attached to a solid support and the solid support itself possess available unsaturated groups, such as alkenyl groups, wherein the anion exchange group-containing monomer becomes covalently bonded to the solid support by a free radical reaction between available unsaturated groups. Because both the anion exchange functional group-containing monomers and synthetic resin support particles of the present invention may possess such available unsaturated groups, the described method

may be employed to covalently attach the anion exchange groups to synthetic resin support particles.

As described above, the ligand exchange and anion exchange functional groups may be attached to the synthetic resin support particles by methods which are known in the art. Either functional group type may be attached to the synthetic stationary phase first followed subsequently by attachment of the other or the different types of functional groups may be attached to the stationary phase simultaneously. Preferably, however, the ligand exchange sites are first attached to a sulfonated support particle followed by attachment of the anion exchange groups (or precursors thereof) by latex deposition onto the surface of the particle.

After attaching the ligand exchange sites and anion exchange functional groups to the synthetic resin support particles as described above to form the bifunctional resin composition, the resin composition may then be formed into beds which are employed for liquid chromatography, whereby a sample stream may flow through the bed of resin, thereby allowing separation of carbohydrates and/or organic acids in the sample stream. These chromatogaphy beds may be formed into many shapes and sizes, preferably into chromatography columns, where the resin is packed into columns using well known methodology. For example, U.S. Patent No. 4,351,909 discloses methods for preparing chromatography columns where the agglomeration of latex polymers onto synthetic resin support particles or covalent bonding thereto is performed before the column is pressure packed with those particles. On the other hand, chromatography columns may be pressure packed with synthetic resin support particles followed by the subsequent agglomeration of the latex polymer thereon. See U.S. Patent Nos. 4,438,047 and 4,351,909.

B. **Functional characteristics of the bifunctional liquid chromatography resin**

The presently described bifunctional liquid chromatography resin composition represents a combination of a ligand exchange separation mechanism for carbohydrates and an anion exchange separation

mechanism for organic acids and is, therefore, capable of simultaneously separating both carbohydrates and organic acids in a mixture thereof. For carbohydrate separation at the metal cation-containing ligand exchange sites, depending upon the metal cation employed, a certain number of water molecules bind to the metal ion. When a carbohydrate mixture is introduced into the mobile phase, the sample components will displace some of the bound water molecules, thereby forming a donor-acceptor complex. The stability of the complex depends upon a number of parameters including, for example, carbohydrate structure and type of metal cation employed. As a result, those carbohydrates which exhibit greater complex stability with the metal cation will elute from the bifunctional resin material after those carbohydrates having low complex stability with the metal cation employed. See Stefansson and Westerlund (1996), *supra*. Separation of organic acids at the available anion exchange sites occurs through the standard anion exchange mechanism which is well known in the art.

The eluent employed with the bifunctional resin compositions of the present invention is a basic hydroxide-containing eluent which may be potassium hydroxide, sodium hydroxide, and the like, preferably matching eluent cation and resin form such as potassium form resin and potassium hydroxide eluent. In the initial stage of separation from the bifunctional resin composition of the present invention, the concentration of the hydroxide-containing eluent employed is relatively low, typically in the range of about 0.1 to 10 mM, preferably from about 0.5 to 5 mM and more preferably from about 1 to 4 mM. At these low eluent concentrations, the carbohydrates in the eluent are effectively non-ionized whereas the organic acids in the eluent are fully ionized. As such, the non-ionized carbohydrates are able to pass through the electrically charged latex coating on the surface of the synthetic resin support particles and exchange at the internally located ligand exchange sites. If the carbohydrates are polysaccharides, the charge on the polysaccharide may be high enough to interact with the anion exchange sites rather than the ligand exchange sites. The ionized organic acids, however, are excluded

from the ligand exchange resin by Donnan exclusion and, therefore, are available to exchange only at the available anion exchange sites in the outer latex coating of the resin composition. At low eluent concentrations (i.e., about 0.1 to 10 mM), monovalent organic acids are able to elute from the anion exchange sites and enter the column flowthrough whereas divalent and trivalent remain attached to the resin composition. Divalent and trivalent organic acids may then be eluted from the bifunctional resin composition, respectively, by gradually increasing the concentration of the eluent, typically starting from about 10 mM and proceeding up to about 100 mM, preferably 80 mM and more preferably 50 mM. However, because the ligand exchange mechanism may be rendered ineffective at eluent concentrations of about 10 mM and above, the initial step of the separation must be performed at low eluent concentrations, so as to allow effective carbohydrate and monovalent organic acid separation, before the eluent concentration is increased to elute the divalent and trivalent organic acids from the column.

Another advantage to employing a basic eluent in the initial stages of the separation is that weakly basic solutions accelerate mutarotation of the carbohydrate species into their different anomers. Therefore, the weakly basic eluent eliminates the multiple anomer peaks which are typically observed when water is employed as the eluent. Thus, the chromatography need not be performed at elevated temperatures for the purpose of reducing or eliminating the detection of multiple anomer peaks.

Once the carbohydrates and organic acids have been separated by the bifunctional resin composition, the presence of those molecules in the chromatography bed effluent can be detected in a variety of different ways. For example, refractive index is one of the most commonly applied detection mechanism for the determination of carbohydrates, wherein a refractive index detector may be placed in fluid communication with the resin bed so as to analyze the effluent containing the combined carbohydrate and organic acid separation medium. By "fluid communication" is meant that one component (such as a resin bed) is connected to another component (such as a suppressor or a detector) in

such a manner that the fluid eluting from the resin bed moves into the suppressor or detector. In a preferred embodiment, the resin bed effluent is passed through a suppressor prior to being passed through a refractive index detector so as to eliminate refractive index bias caused by variable eluent concentrations. Refractive index detectors and suppressors and their mechanisms of action are well known in the art. Refractive index detectors may also be employed for the analysis of organic acids, however, other known detection means are more sensitive. An example of a refractive index detector which finds use herein is the refractive index detector by Shimadzu, Model No. RID-6A.

For detection of the separated organic acids, a conductivity detector may be employed in series with a refractive index detector, both detectors optionally being placed downstream from a suppressor. Conductivity detectors and their methods of use are also well known in the art and as described in U.S. Patent No. 3,920,397. An example of a conductivity detector which finds use herein is Dionex Corporation's Model No. CD-20.

Another combination which finds use for detection purposes in the present invention is a combination of an amperometric detector for determination of carbohydrates and a conductivity detector for determination of the organic acids, the amperometric detector being upstream from the conductivity detector and both being placed downstream from a suppressor. Amperometric detectors and their methods of use are also well known in the art.

C. <u>Uses of the bifunctional liquid chromatography resin compositions</u>

The bifunctional liquid chromatography resin compositions of the present invention are primarily useful for the simultaneous separation of carbohydrates and organic acids from mixtures thereof in a single chromatography run. As such, the novel bifunctional resins of the present invention find use for a variety of different applications including, for example, in the efficient analysis of carbohydrates and organic acids in food and beverage products.

The bifunctional chromatographic resin compositions of the present invention may be employed in methods for separating carbohydrates and organic acids from a mixture thereof which may be a complex mixture. To do so, the bifunctional resin compositions of the present invention are packed into chromatography columns for use in liquid chromatography. The columns are then contacted with a fluid mixture of various carbohydrates and organic acids which are present in an eluent and separation of the carbohydrate species in the mixture is allowed to occur at the ligand exchange sites of the bifunctional resin whereas organic acid separation occurs at the anion exchange sites of the bifunctional resin composition.

Further details of the invention are illustrated in the following non-limiting examples.

EXAMPLE I - Separation and Analysis of Carbohydrates and Organic Acids in Various Beverages Using a Potassium Based Bifunctional Resin.

The bifunctional liquid chromatography resin employed herein was prepared, packed into a chromatography column and used to separate and analyze carbohydrates and organic acids in various beverages including apple juice, orange juice, cranberry juice, grapefruit juice, swiss mocha, grape juice, red grape must, white grape must, and merlot wine. Specifically, neutral styrene divinylbenzene resin support particles were sulfonated to produce a hydrogen form thereof and then filtered with potassium hydroxide to create the potassium metal-containing ligand exchange functional groups attached to the resin support particles (i.e., the ligand exchange resin). Ten grams of Dionex Corporation AS11 latex (latex which comprises quaternary ammonium anion exchange functional groups) was then diluted to 100 grams in 0.5 M KCl and was added to 25 grams of the ligand exchange resin which had been previously diluted to 40 grams in 0.5 M KCl. The mixture was stirred for 5 min., filtered and then washed three times with 0.5 M KCl. The resin was then diluted to 40 grams with 0.5 M KCl and packed into 9x250 mm liquid chromatography column for use.

For liquid chromatography analysis, the columns were run at 38°C with an injection volume of 10 μl and a flow rate of 1 ml/min. The eluent employed was potassium hydroxide at 1.8 mM for the first 13 minutes, 30 mM for the next 9 minutes and then to 65 mM. The chromatography column was connected to a Dionex Corporation Anion Self-Regenerating Suppressor, a Dionex Corporation CD-20 Conductivity Detector for detection of organic acids and a Shimadzu RID-6A Refractive Index Detector for detection of carbohydrates.

All of the fruit juice samples tested were diluted 1:100 with distilled water whereas the wine sample was diluted 1:30, the grape must samples diluted 1:200 and the swiss mocha sample diluted 1:400 prior to injection onto the column. The wine sample was additionally pretreated with a Dionex Corporation ONGARD-RP sample pretreatment cartridge. The results obtained from these experiments are presented in Figs. 4-12.

As shown in Figs. 4-12, a bifunctional resin composition comprising potassium-based ligand exchange functional groups and anion exchange functional groups is capable of efficiently and simultaneously separating both the carbohydrate and organic acid components of the beverages tested.

EXAMPLE II - Separation and Analysis of Carbohydrates and Organic Acids in a Synthetic Mixtures Thereof Using a Potassium-Based Bifunctional Resin.

A synthetic mixture of various carbohydrates, organic acids and ethanol was prepared and analyzed as described in Example I above. The synthetic mixture contained 100 mg/L sucrose, 100 mg/L maltose, 100 mg/L glucose, 100 mg/L fructose, 200 mg/L ethanol, 10 mg/L quinic acid, 10 mg/L lactic acid, 10 mg/L acetic acid, 5 mg/L formic acid, 10 mg/L pyruvic acid, 10 mg/L succinic acid, 10 mg/L tartaric acid, 10 mg/L oxalic acid and 10 mg/L citric acid. The results of this experiment are present in Fig. 13.

As shown in Fig. 13, a bifunctional resin composition comprising potassium-based ligand exchange functional groups and anion exchange

functional groups is capable of efficiently and simultaneously separating the carbohydrate, organic acid and ethanol components of the synthetic mixture tested.

EXAMPLE III - Separation and Analysis of Carbohydrates and Organic Acids in a Synthetic Mixture Thereof Using a Cesium-Based Bifunctional Resin.

A bifunctional resin was produced as described in Example I above except that the cation exchange metal cation attached to the resin was cesium (by contacting the hydrogen form of the resin with 0.5 M CsCl) instead of potassium. The resin was packed into chromatography columns and employed for the separation of carbohydrates and organic acids in a synthetic mixture thereof, wherein the eluent was CsOH (2.5 mM for the first 12 minutes and then to 30 mM). The synthetic mixture contained 20 mg/L of the following: sucrose, maltose, glucose, fructose, quinic acid, lactic acid, acetic acid, formic acid, pyruvic acid, succinic acid, malic acid, tartaric acid, oxalic acid and fumaric acid. Detection of the components in the eluent was performed by refractive index detection. The results of this experiment are present in Fig. 14.

As shown in Fig. 14, a bifunctional resin composition comprising cesium-based ligand exchange functional groups and anion exchange functional groups is capable of efficiently and simultaneously separating the carbohydrate and organic acid components of the synthetic mixture tested.

The foregoing description details specific methods which can be employed to practice the present invention. Having detailed such specific methods, those skilled in the art will well enough know how to devise alternative reliable methods at arriving at the same information in using the fruits of the present invention. Thus, however, detailed the foregoing may appear in text, it should not be construed as limiting the overall scope thereof; rather, the ambit of the present invention is to be determined only by the lawful construction of the appended claims. All documents cited herein are expressly incorporated by reference.

WHAT IS CLAIMED IS:

1. A bifunctional chromatography resin composition capable of simultaneously separating both carbohydrates and organic acids in a sample stream comprising said carbohydrates and organic acids which is flowing through said resin composition, said bifunctional resin composition comprising:

(a) synthetic resin support particles;

(b) ligand exchange functional groups attached to said synthetic resin support particles, wherein said ligand exchange functional groups comprise one or more metal cations, and

(c) anion exchange functional groups attached to said synthetic resin support particles.

2. The bifunctional resin composition according to Claim 1, wherein said ligand exchange functional groups are capable of interacting with at least one carbohydrate present in said sample stream flowing through said resin composition and thereby delaying the elution of said at least one carbohydrate from said resin composition.

3. The bifunctional resin composition according to Claim 1, wherein said anion exchange functional groups are capable of interacting with at least one organic acid present in said sample stream flowing through said resin composition and thereby delaying the elution of said at least one organic acid from said resin composition.

4. The bifunctional resin composition according to Claim 1, wherein said metal cations are selected from the group consisting of potassium cations, calcium cations, sodium cations, cesium cations, strontium cations, barium cations, and mixtures thereof.

5. The bifunctional resin composition according to Claim 1, wherein said metal cations are potassium cations.

6. The bifunctional resin composition according to Claim 1, wherein said ligand exchange functional groups comprise two or more different metal cations.

7. The bifunctional resin composition according to Claim 1, wherein ligand exchange functional groups are attached to internal surfaces defined by pores in said synthetic resin support particles.

8. The bifunctional resin composition according to Claim 1, wherein said anion exchange functional groups comprise quaternary ammonium groups.

9. The bifunctional resin composition according to Claim 1, wherein said anion exchange functional groups are attached to said synthetic resin support particles by deposition from a latex comprising said anion exchange functional groups.

10. The bifunctional resin composition according to Claim 9, wherein said anion exchange functional group-containing latex is attached to said synthetic resin support particles through electrostatic forces.

11. The bifunctional resin composition according to Claim 9, wherein said latex is attached to said synthetic resin support particles through a dispersant material.

12. A chromatography bed for use in liquid chromatography comprising the bifunctional chromatography resin composition according to Claim 1.

13. The chromatography bed according to Claim 12 in fluid communication with a detector for carbohydrates or organic acids eluting from said bed.

14. The chromatography bed according to Claim 13, wherein said detector comprises a refractive index detector

15. The chromatography bed according to Claim 13, wherein said detector comprises a conductivity detector.

16. The chromatography bed according to Claim 13, wherein said detector comprises an amperometric detector.

17. The chromatography bed according to Claim 13, wherein said detector comprises a refractive index detection in series with a conductivity detector.

18. The chromatography bed according to Claim 13, comprising a suppressor in fluid communication with said chromatography bed and said detector and wherein said suppressor is placed between said chromatography bed and said detector.

19. The chromatography bed according to Claim 12 which is a chromatography column.

20. A method of making the bifunctional chromatography resin composition of Claim 1, said method comprising:

attaching metal cations to negatively charged sites within synthetic resin support particles to form ligand exchange sites within said particles; and

attaching anion exchange functional groups to the surface of said synthetic resin support particles to form said bifunctional resin composition.

21. The method according to Claim 20, wherein said step of attaching said metal cations to negatively charged sites within said synthetic resin support particles comprises contacting sulfonated synthetic resin support particles with a solution comprising said metal cation.

22. The method according to Claim 20, wherein said step of attaching said anion exchange functional groups to the surface of said synthetic resin support particles comprises depositing a latex comprising said anion exchange functional groups onto the surface of said synthetic resin support particles.

23. A method for separating a first carbohydrate from a second carbohydrate in a sample stream comprising at least said first and second carbohydrates and wherein said first and second carbohydrates are different, said method comprising:

flowing said sample stream through a chromatography bed comprising the bifunctional resin composition of Claim 1 in the presence of a hydroxide ion containing eluent, whereby said first carbohydrate interacts with ligand exchange sites in said resin composition and thereby elutes off said chromatography bed later than said second carbohydrate.

24. The method according to Claim 23, wherein said hydroxide containing eluent comprises hydroxide ion at a concentration ranging from about 0.01 to 10 mM.

25. The method according to Claim 23, wherein said sample stream further comprises a mixture of organic acids comprising at least first and second organic acids and wherein said first and second organic acids are different, said method further comprising the step of separating said first organic acid from said second organic acid on said resin composition.

26. A method for separating a first organic acid from a second organic acid in a sample stream comprising at least said first and second organic acids and wherein said first and second organic acids are different, said method comprising:

flowing said sample stream through a chromatography bed comprising the bifunctional resin composition of Claim 1 in the presence of a hydroxide ion containing eluent, whereby said first organic acid interacts with anion exchange sites in said resin composition and thereby elutes off said chromatography bed later than said second organic acid.

27. The method according to Claim 26, wherein said hydroxide containing eluent comprises hydroxide ion at a concentration ranging from about 0.01 to 100 mM.

28. The method according to Claim 27, wherein said hydroxide containing eluent initially comprises hydroxide ion at a concentration of 0.01 to 10 mM and wherein the hydroxide ion concentration in said eluent is subsequently increased to about 10.1 mM to 100 mM.

29. The method according to Claim 26, wherein said sample stream further comprises a mixture of carbohydrates comprising at least first and second carbohydrates and wherein said first and second carbohydrates are different, said method further comprising the step of separating said first carbohydrate from said second carbohydrate on said resin composition.

30. The method according to Claim 23 or 26 further comprising the step of detecting the elution of said carbohydrates or organic acids from said chromatography bed.

31. The method according to Claim 30 further comprising the step of suppressing the conductivity of hydroxide ion present in said eluent, wherein said step of suppressing is performed after said step of flowing and before said step of detecting.

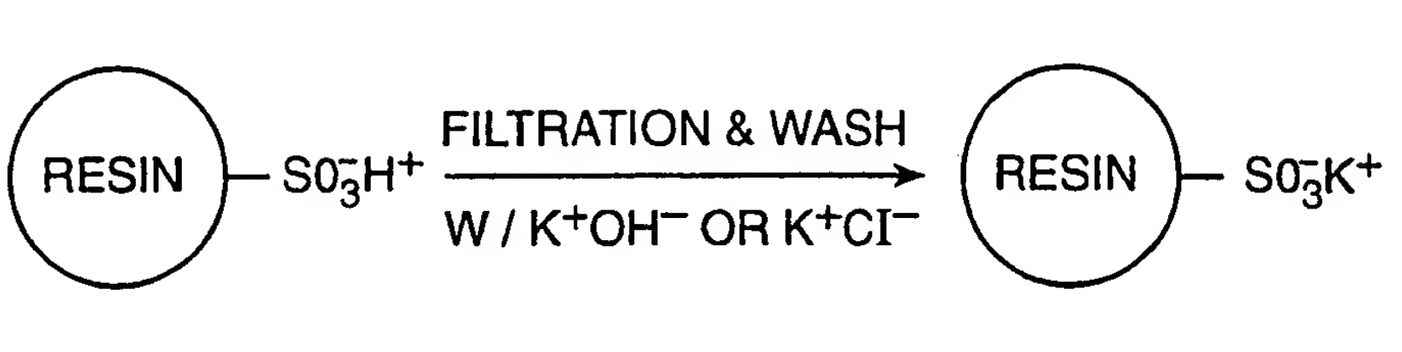


FIG._1

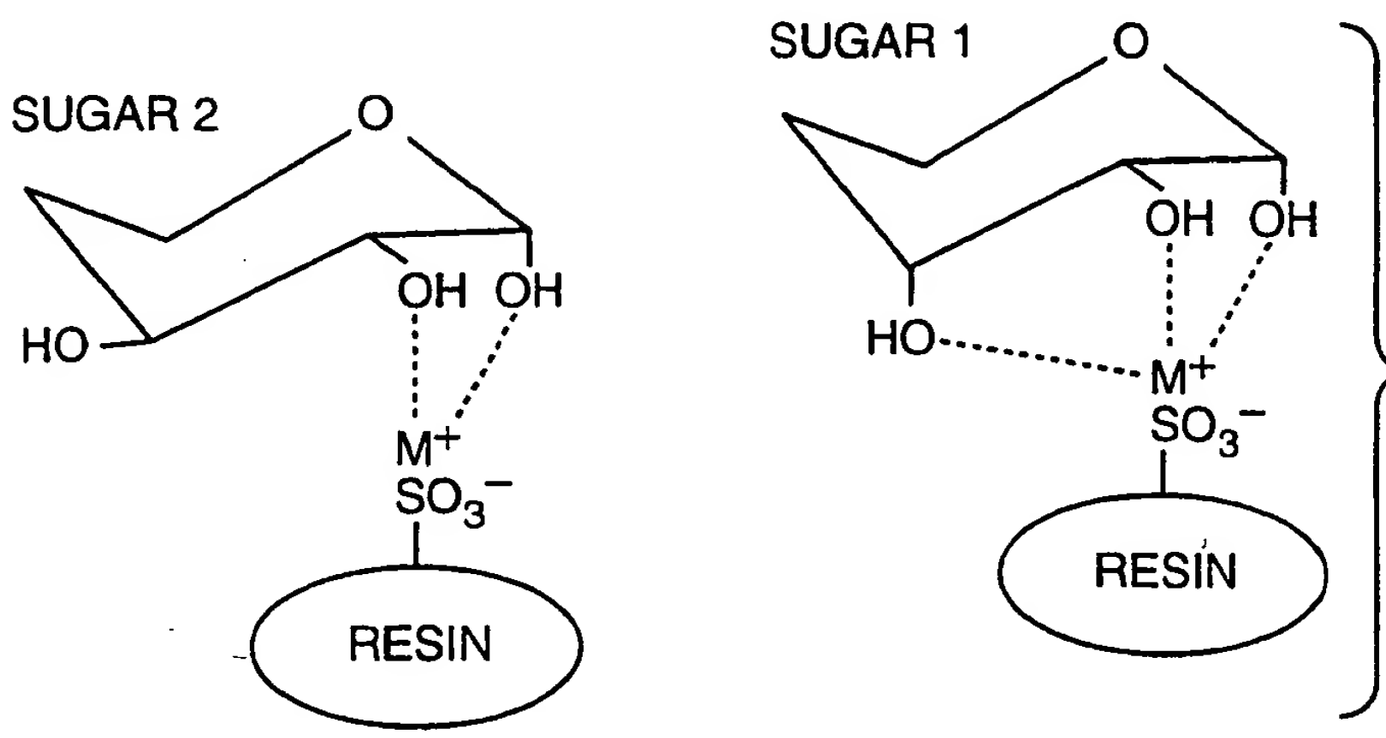


FIG._2

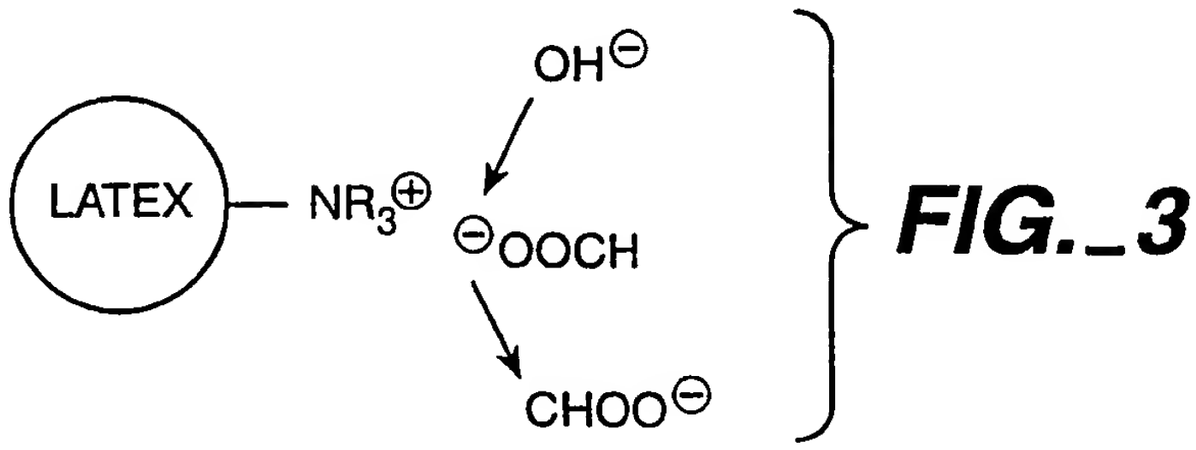


FIG._3

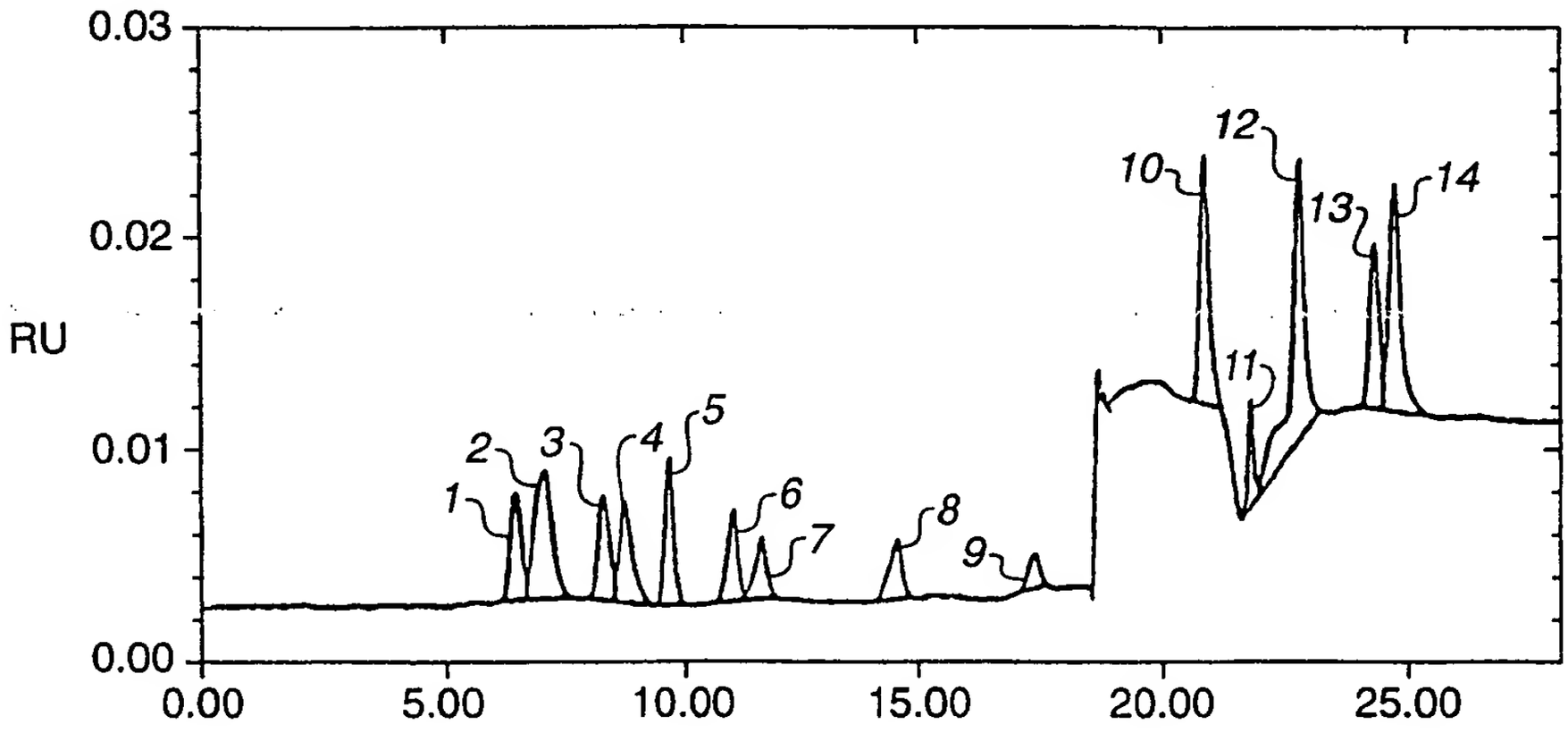
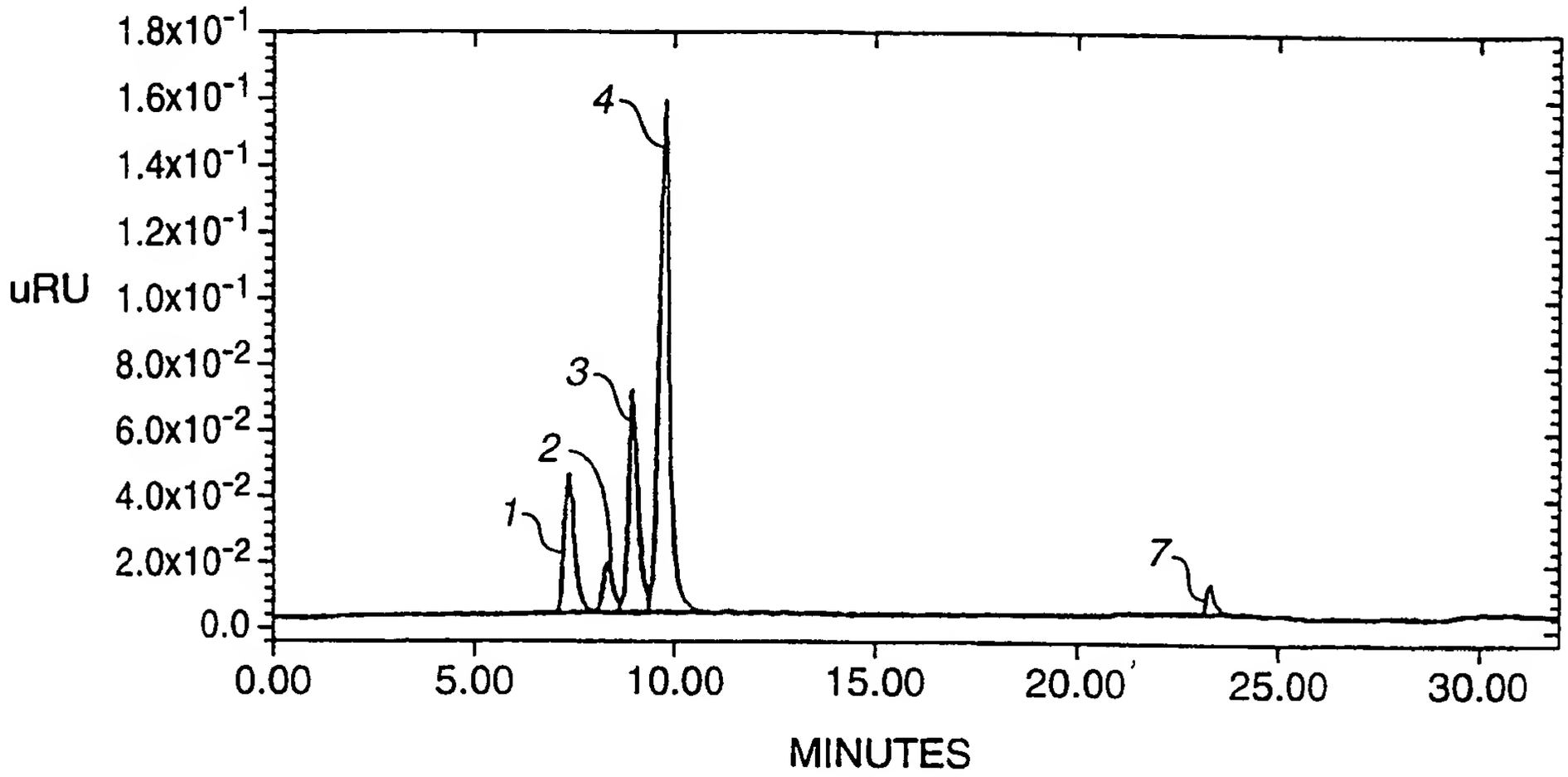


FIG._14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0.0
4
3
2
1
7
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._4A

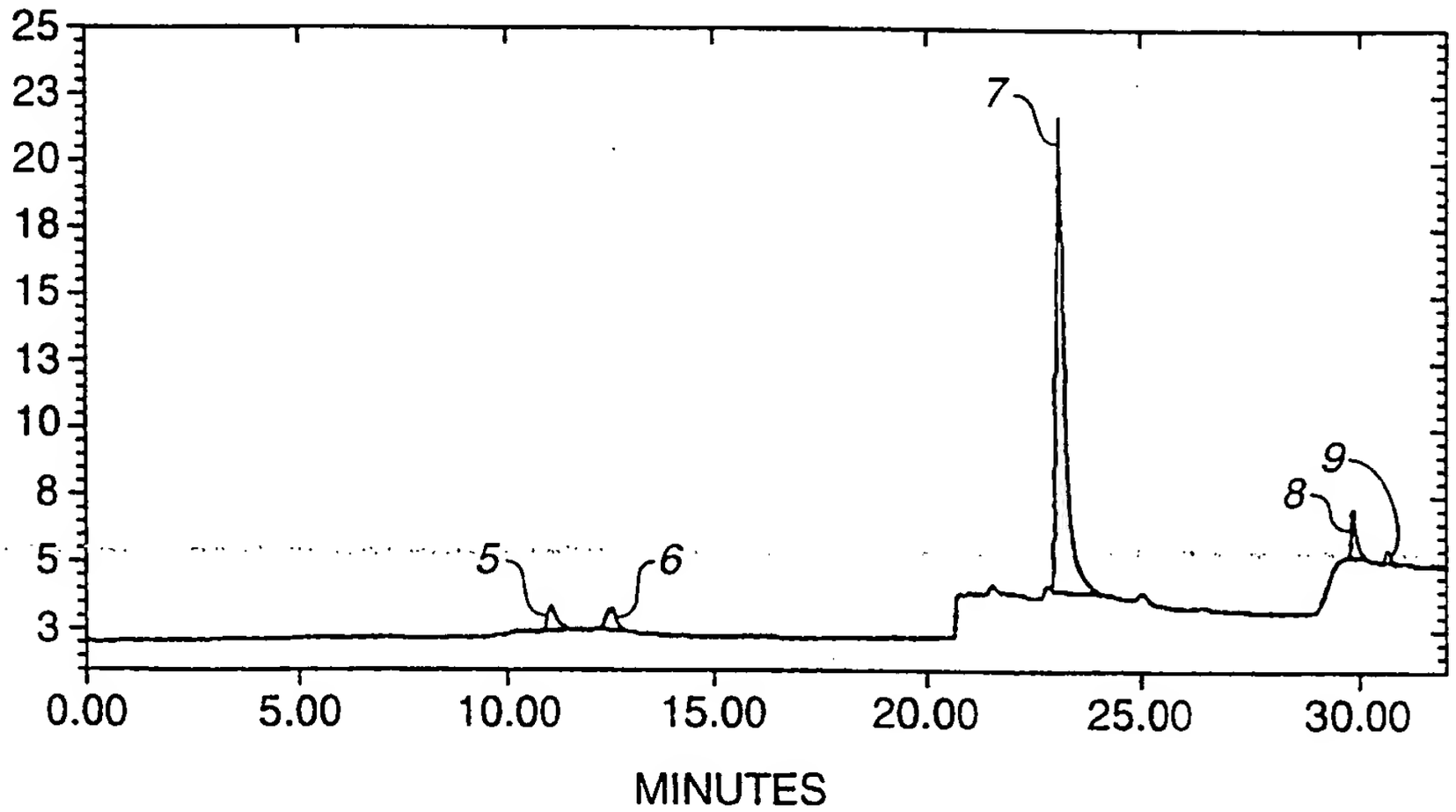

25
23
20
18
µS
15
13
10
8
5
3
7
9
8
5
6
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._4B

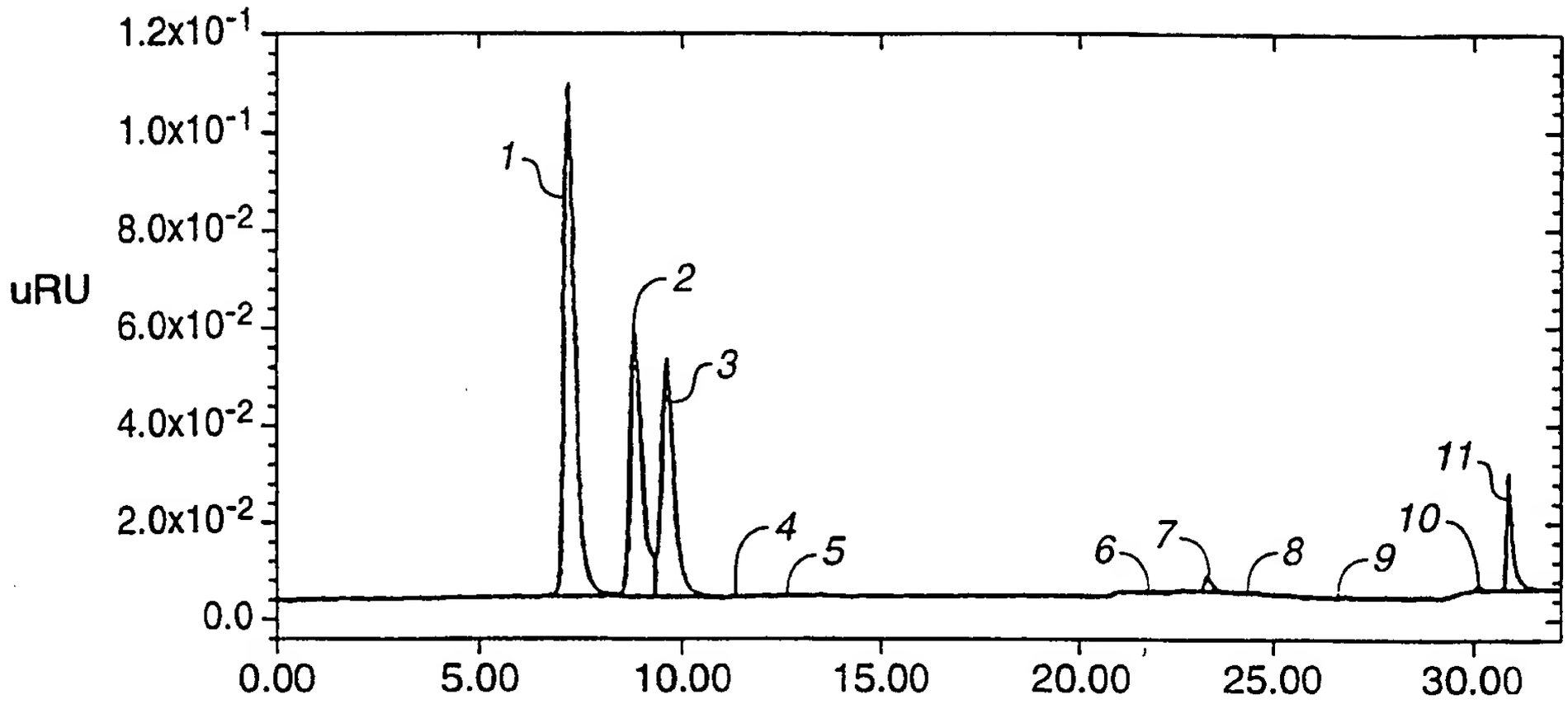

1.2x10-1
1.0x10-1
8.0x10-2
6.0x10-2
4.0x10-2
2.0x10-2
0.0
uRU
1
2
3
4
5
6
7
8
9
10
11
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._5A

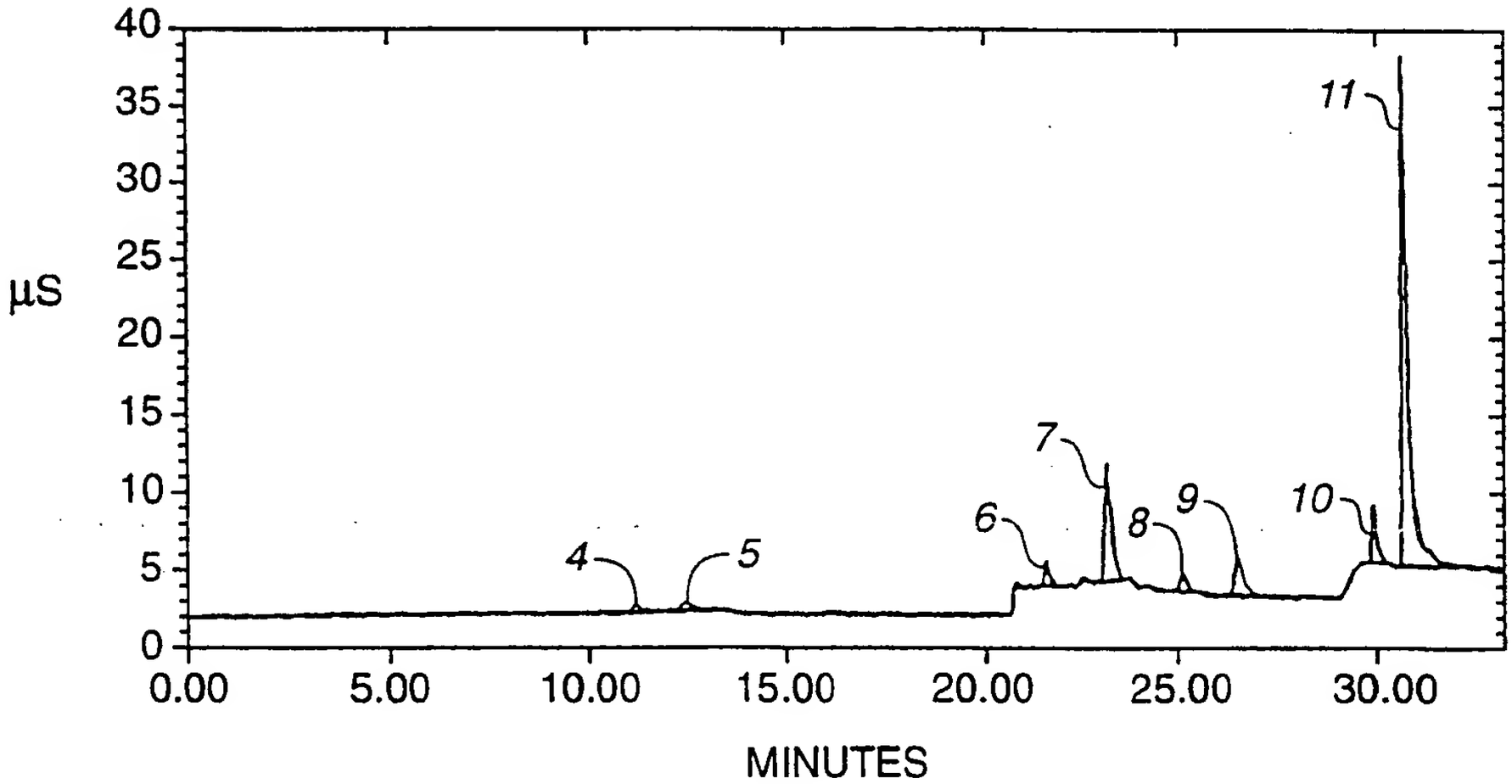

40
35
30
25
20
15
10
5
0
µS
4
5
6
7
8
9
10
11
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._5B

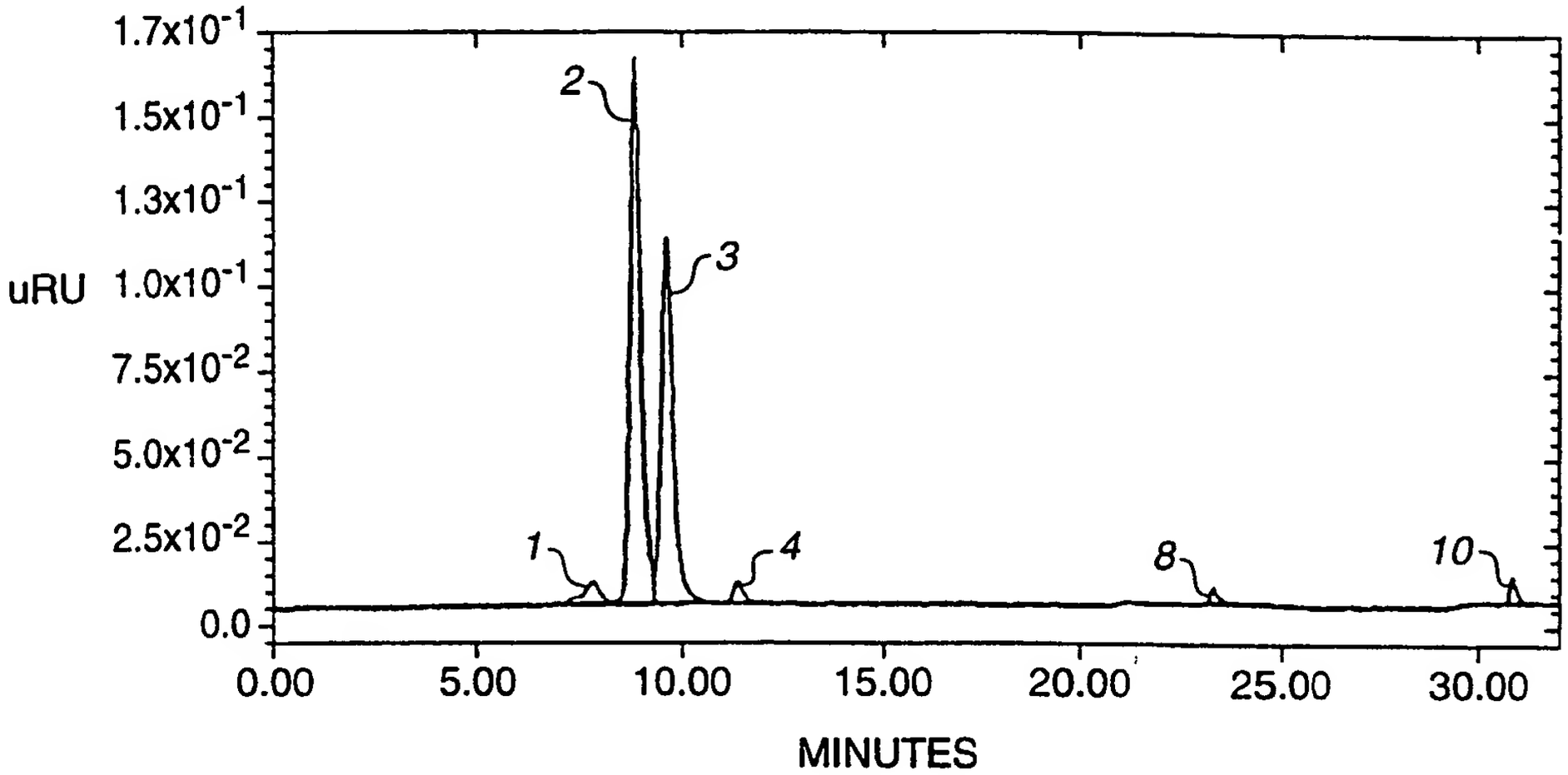

1.7x10-1
1.5x10-1
1.3x10-1
uRU 1.0x10-1
7.5x10-2
5.0x10-2
2.5x10-2
0.0
1
2
3
4
8
10
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._6A

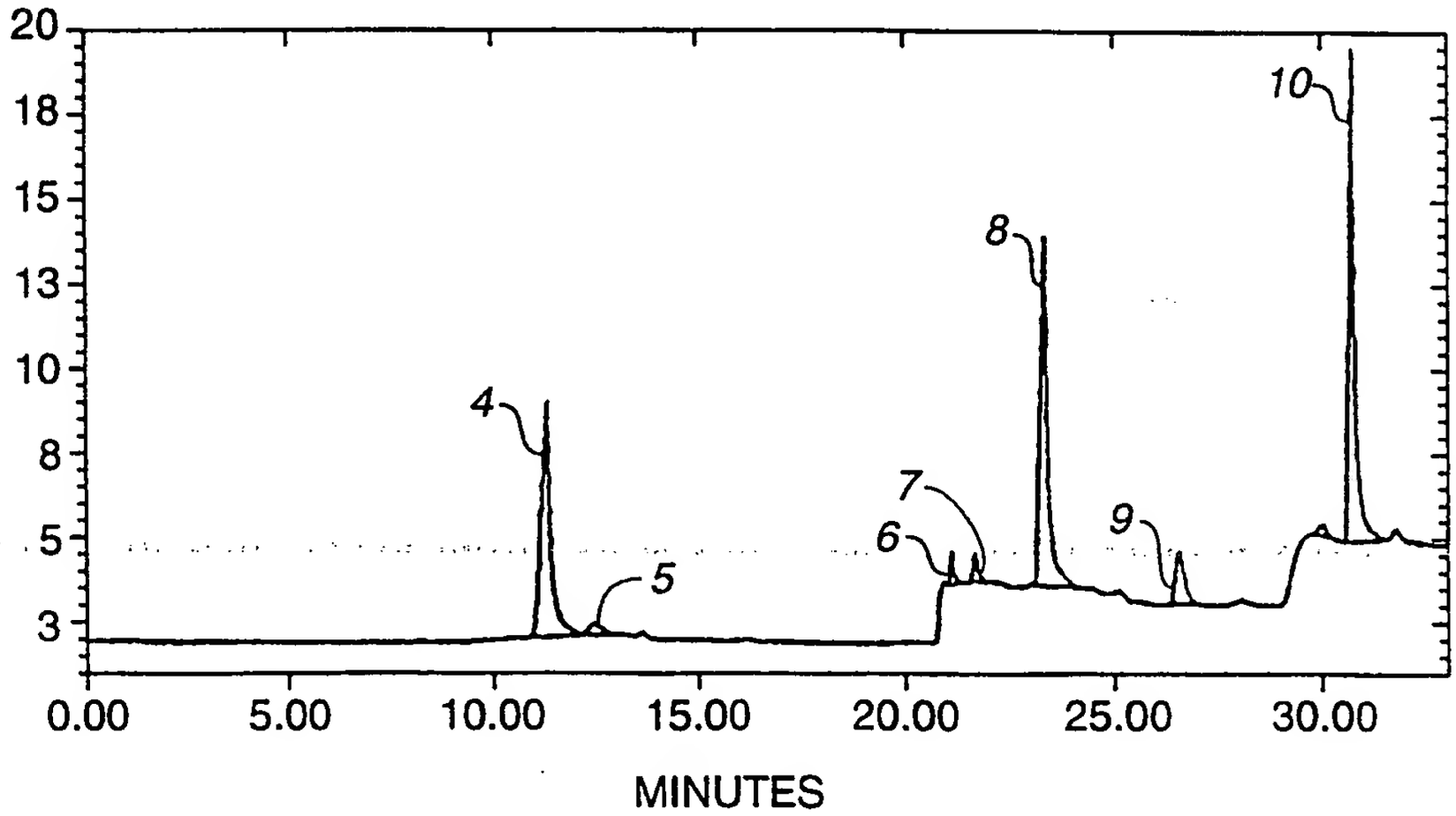
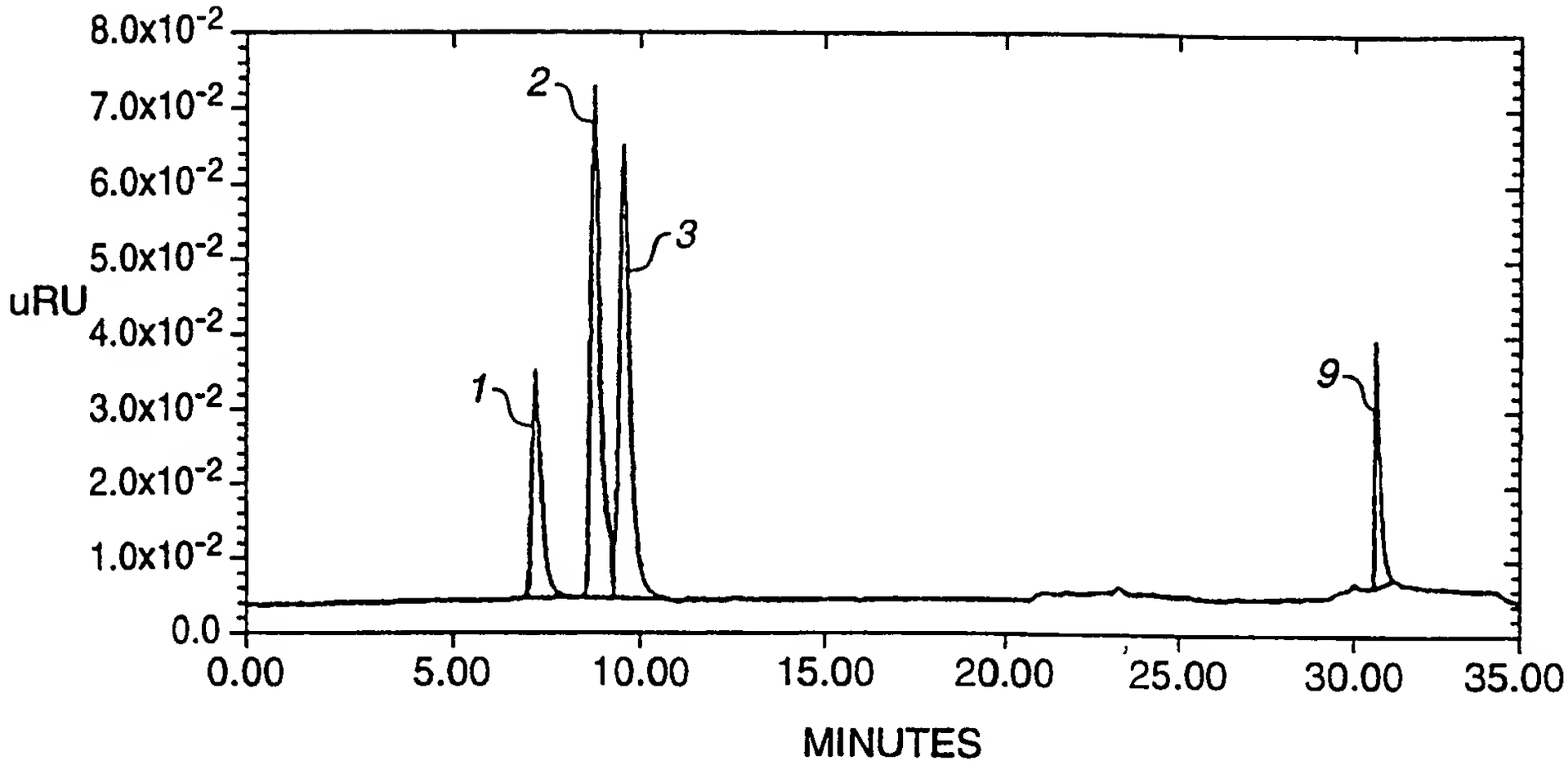

20
18
15
13
µS 10
8
5
3
4
5
6
7
8
9
10
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._6B


8.0x10-2
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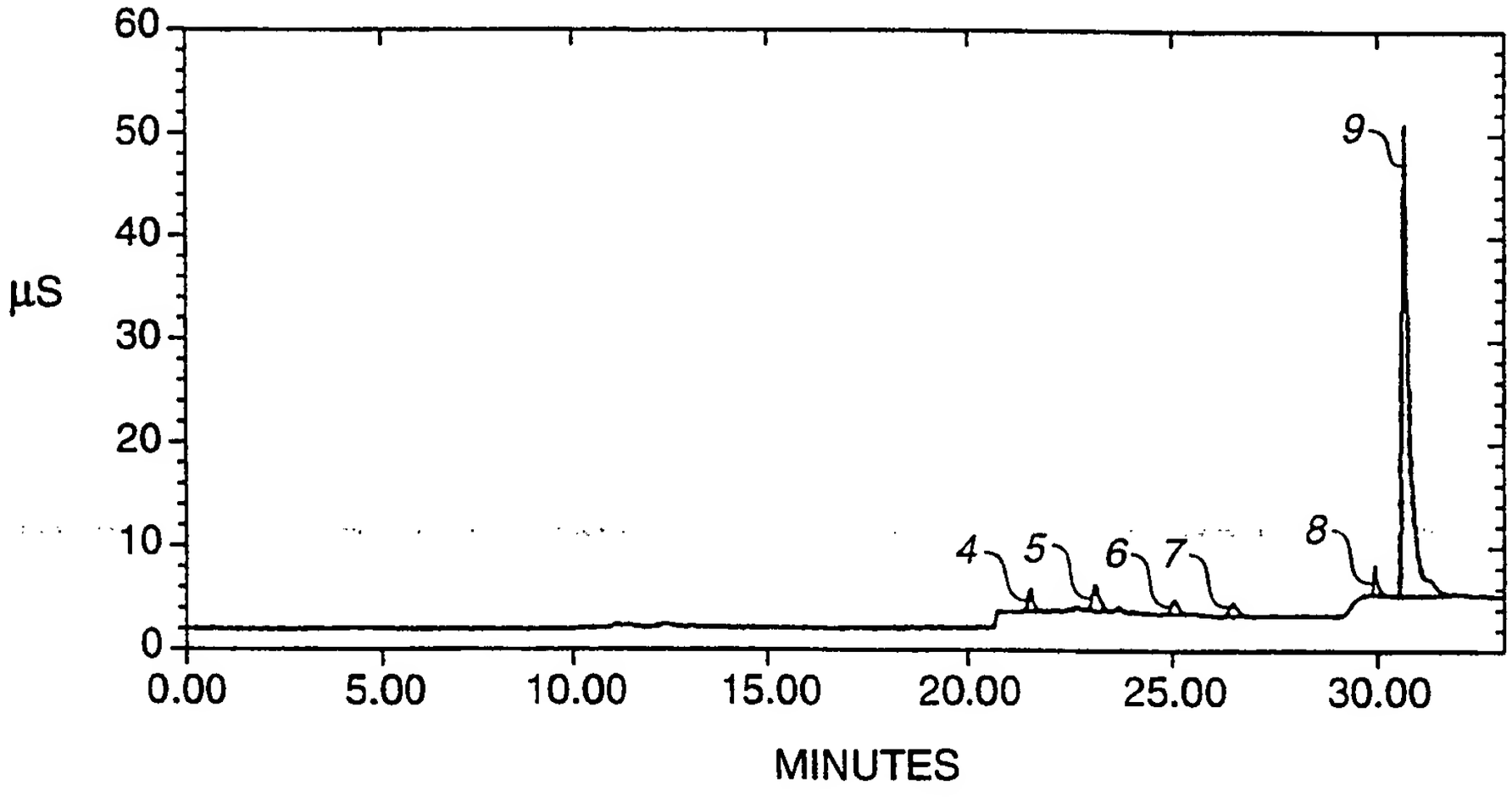
6.0x10-2
5.0x10-2
4.0x10-2
3.0x10-2
2.0x10-2
1.0x10-2
0.0
uRU
1
2
3
9
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
MINUTES

FIG._7A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50
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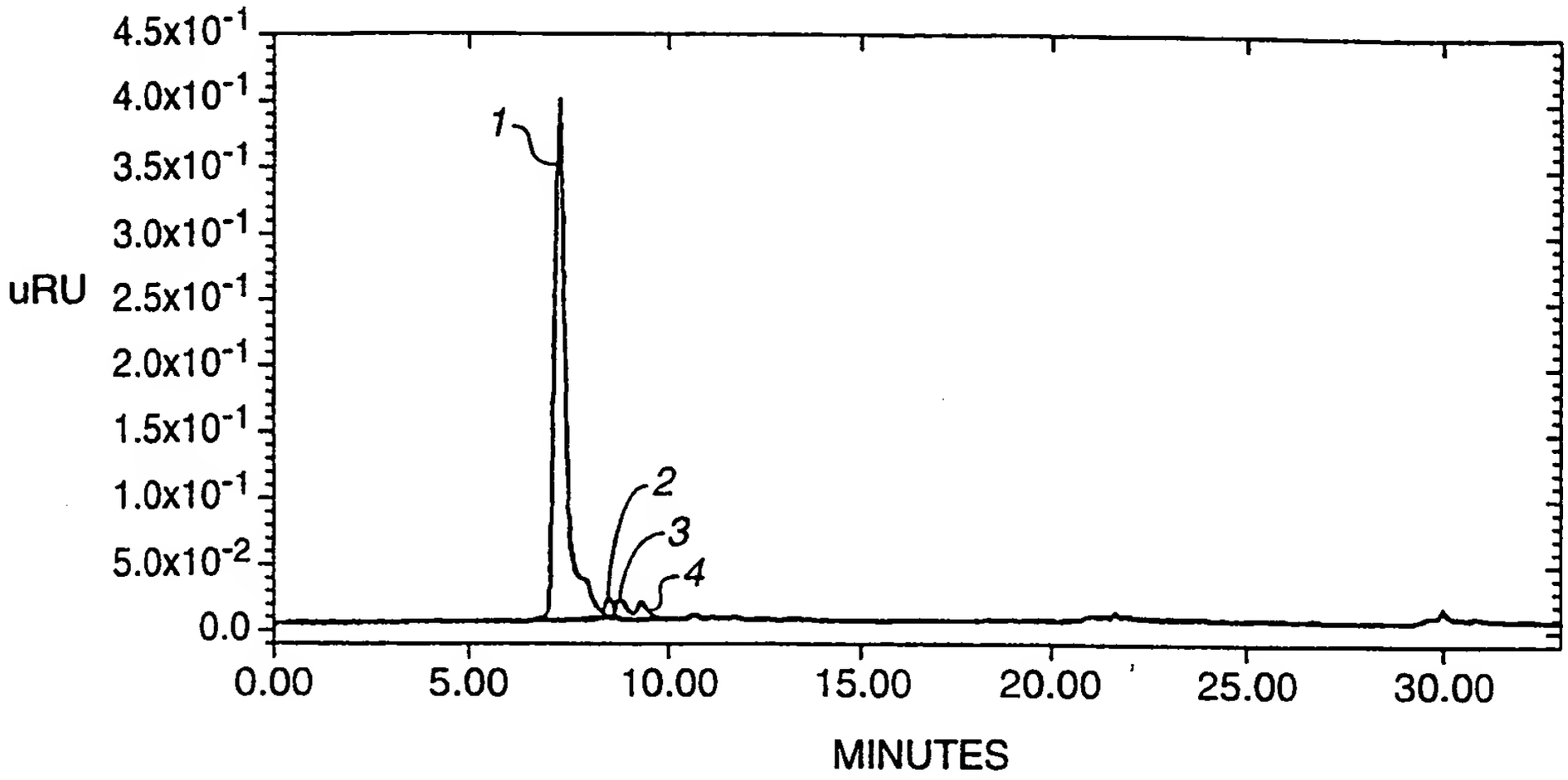
30
20
10
0
µS
9
4
5
6
7
8
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._7B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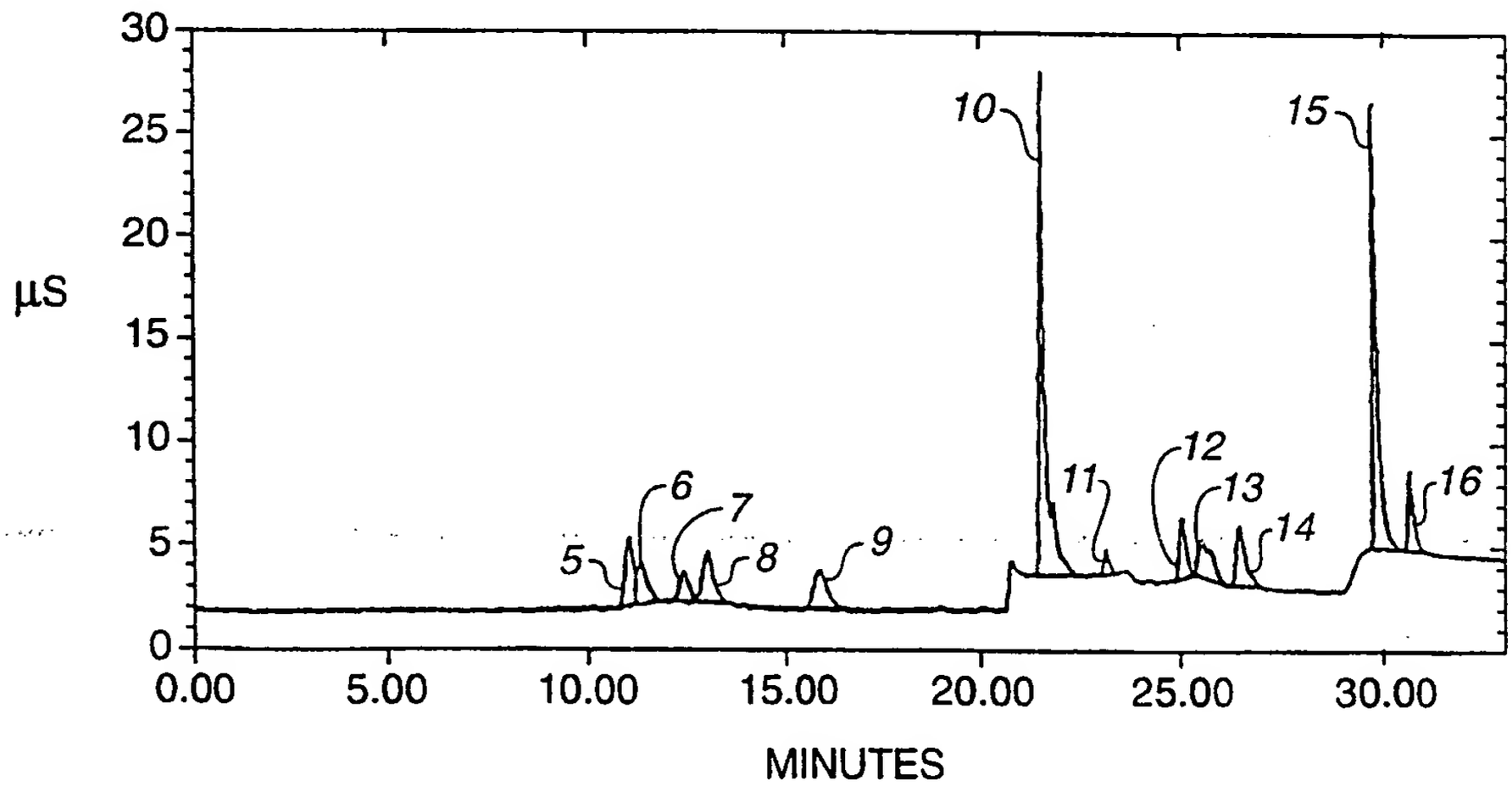
4.0x10-1
3.5x10-1
3.0x10-1
uRU 2.5x10-1
2.0x10-1
1.5x10-1
1.0x10-1
5.0x10-2
0.0
1
2
3
4
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._8A


30
25
20
µS
15
10
5
0
5
6
7
8
9
10
11
12
13
14
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16
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._8B

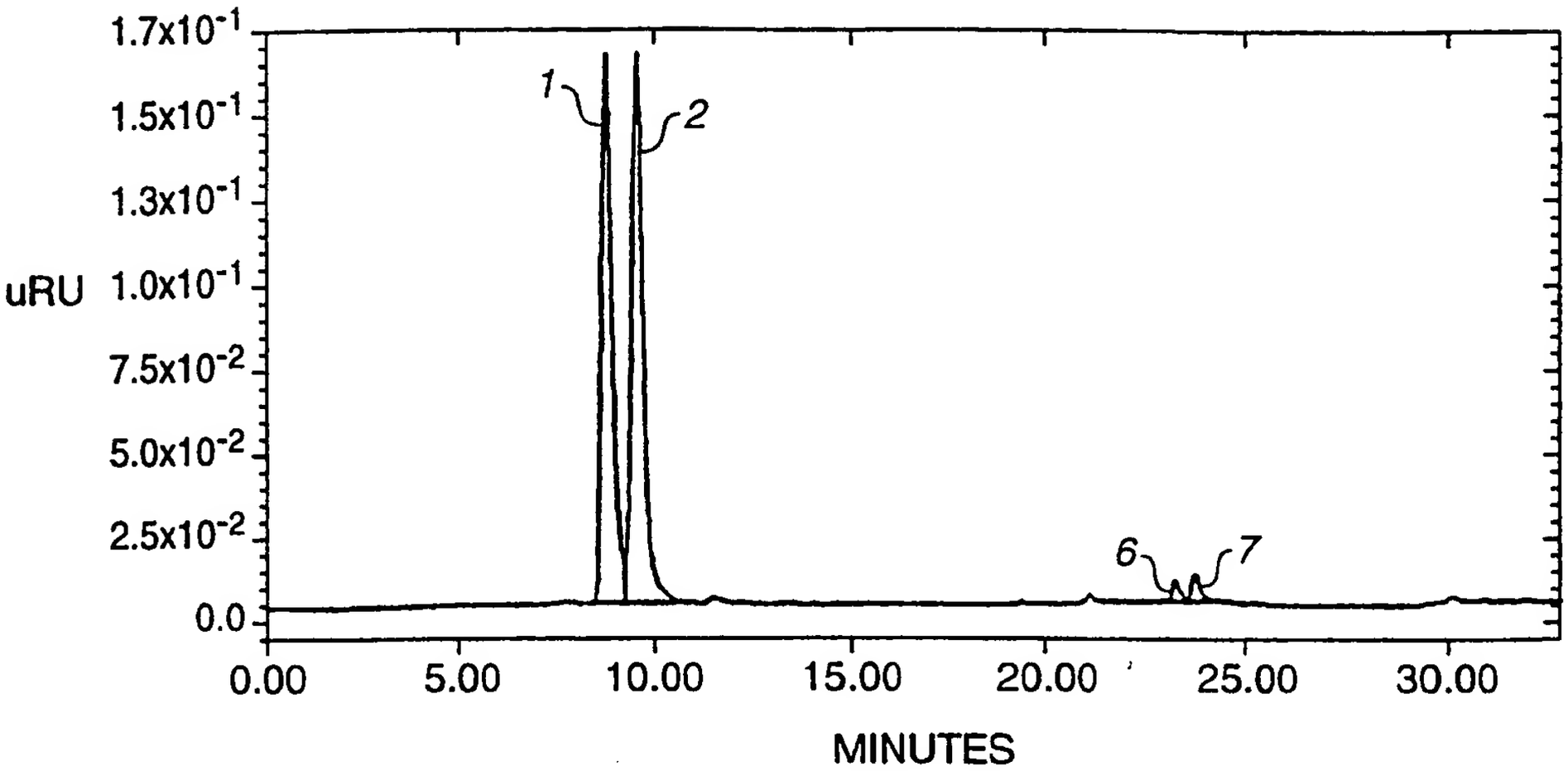
1.7x10-1
1.5x10-1
1.3x10-1
uRU 1.0x10-1
7.5x10-2
5.0x10-2
2.5x10-2
0.0
1
2
6
7
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._9A

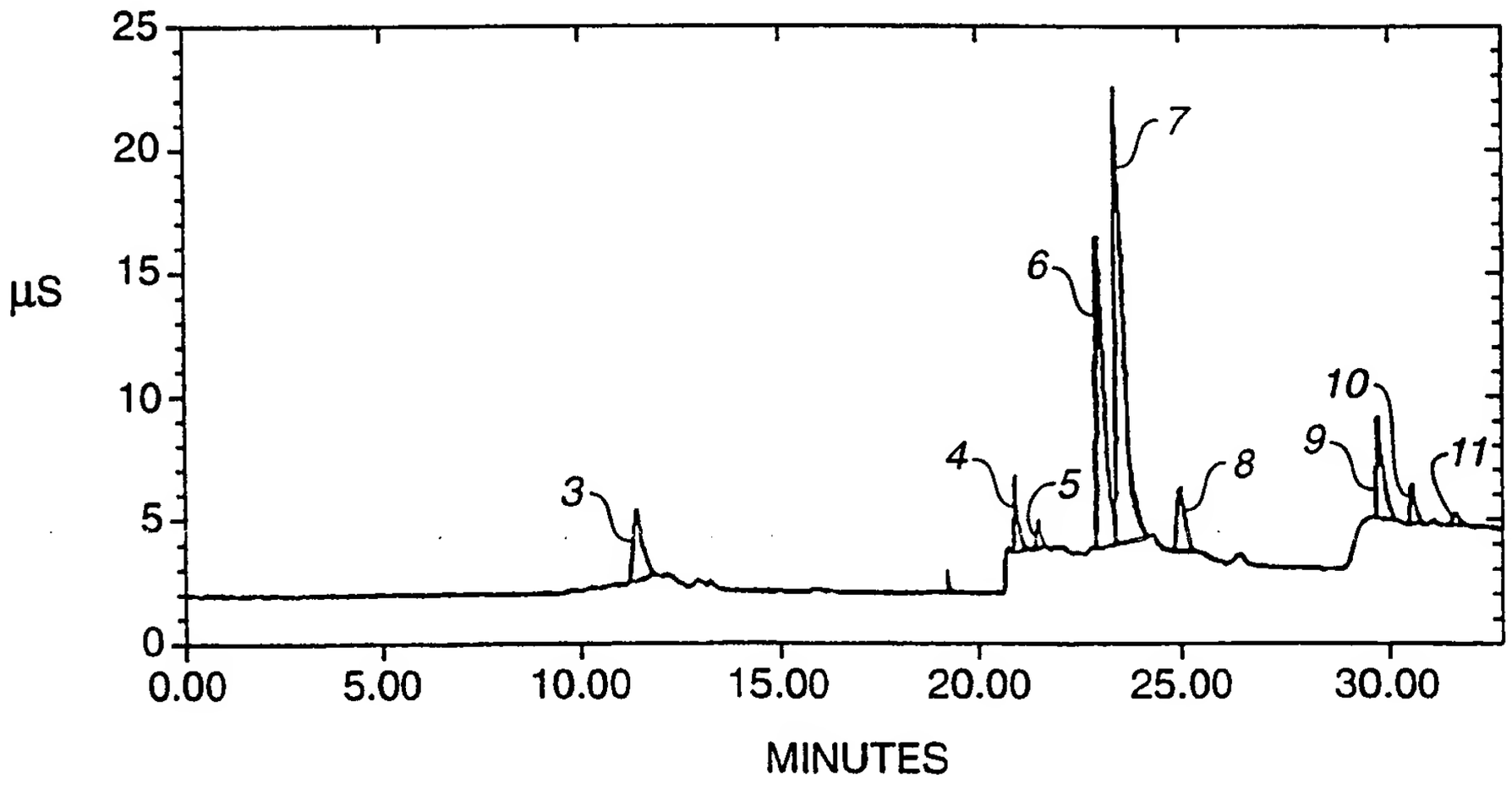
25
20
15
10
5
0
µS
3
4
5
6
7
8
9
10
11
0.00
5.00
10.00
15.00
20.00
25.00
30.00
MINUTES

FIG._9B

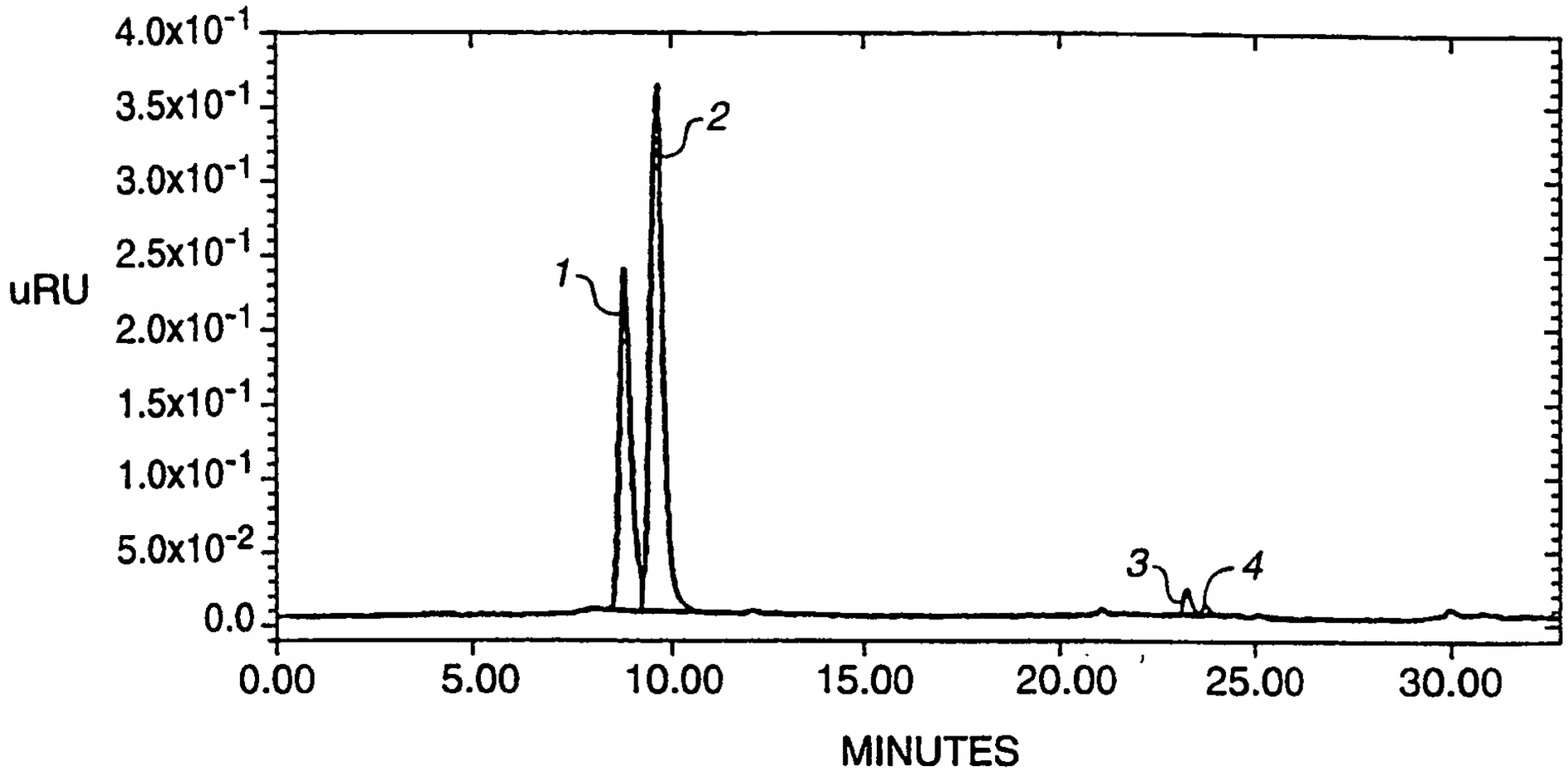

FIG._10A

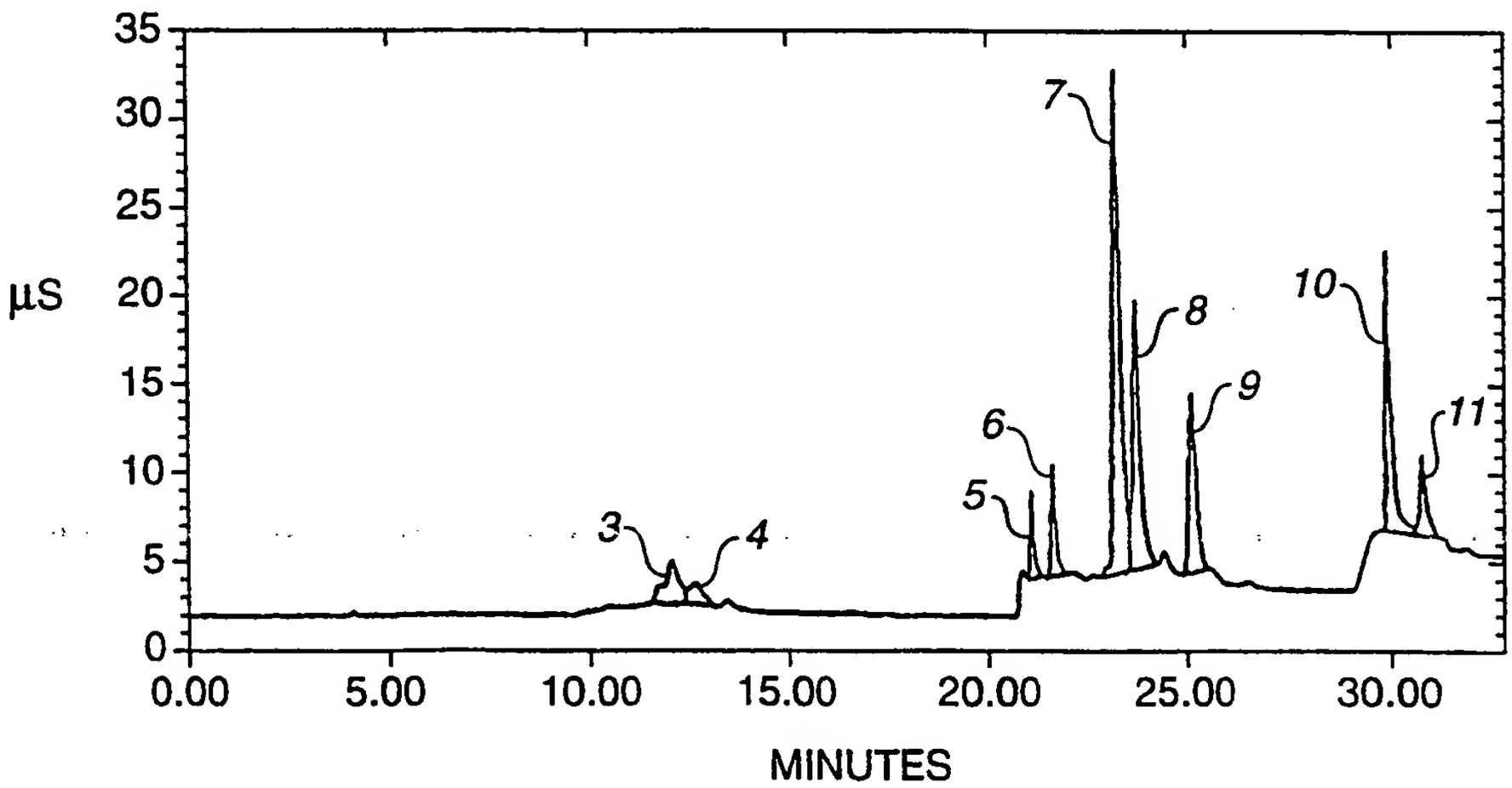

FIG._10B

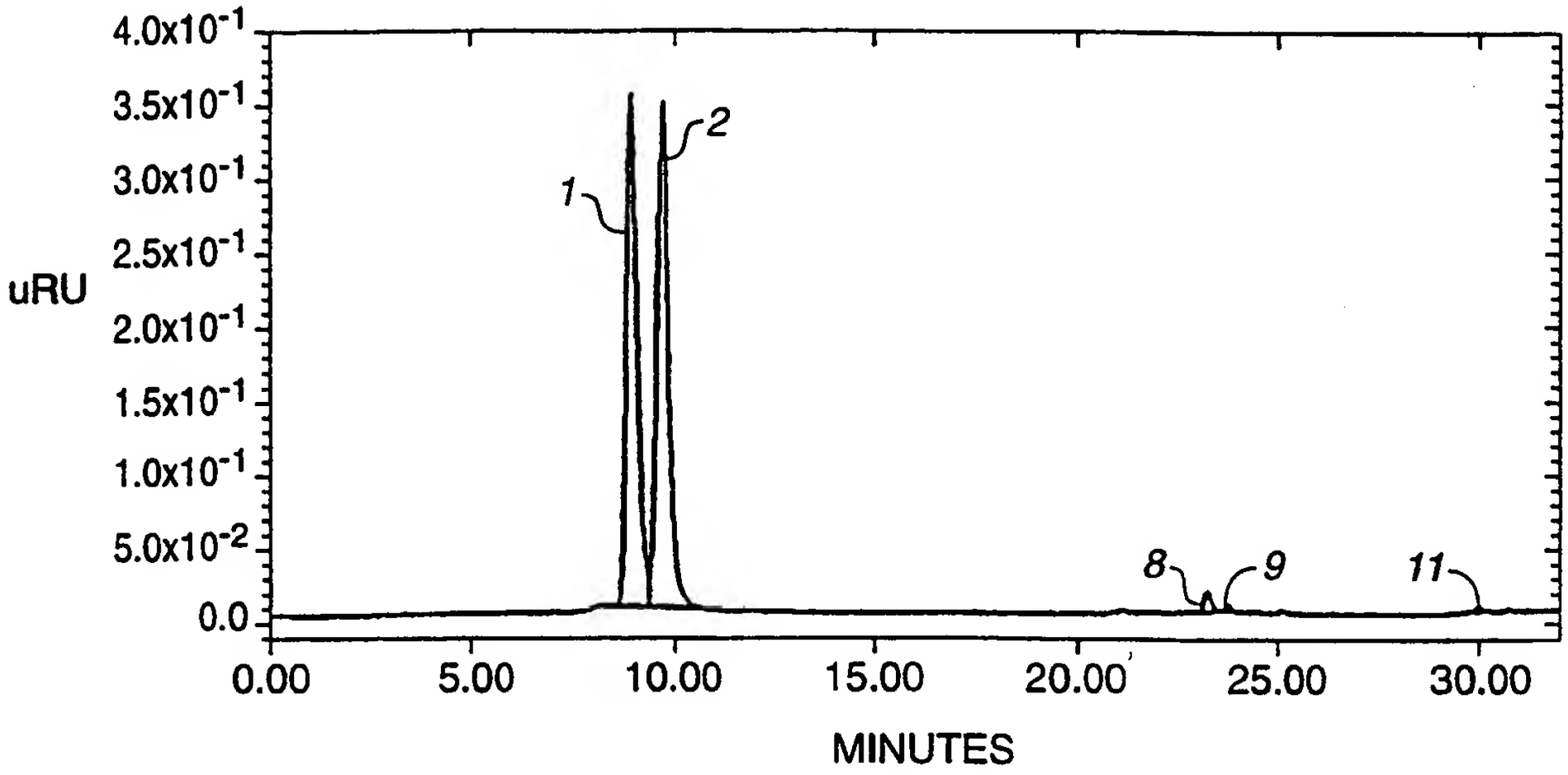

FIG._11A

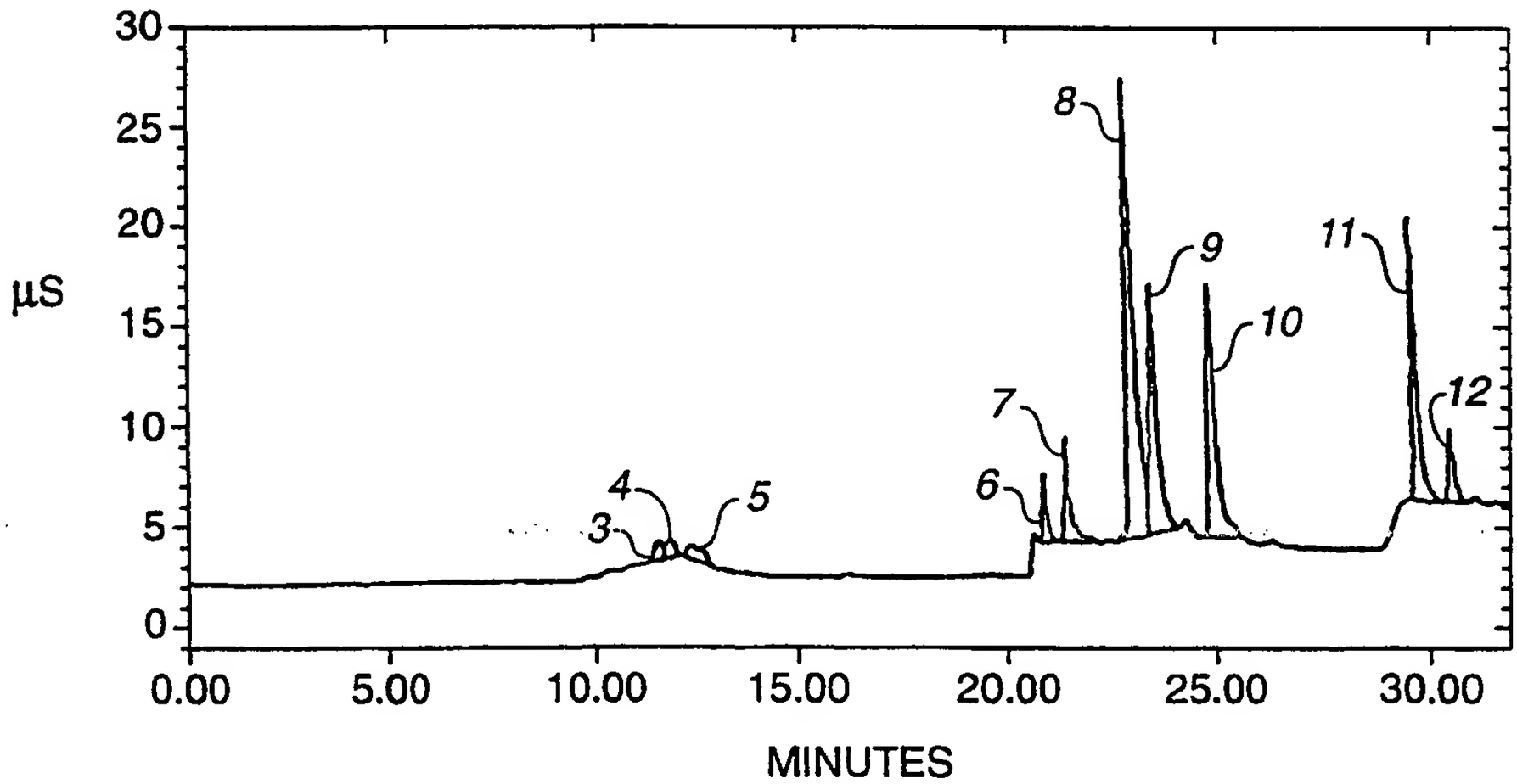

FIG._11B

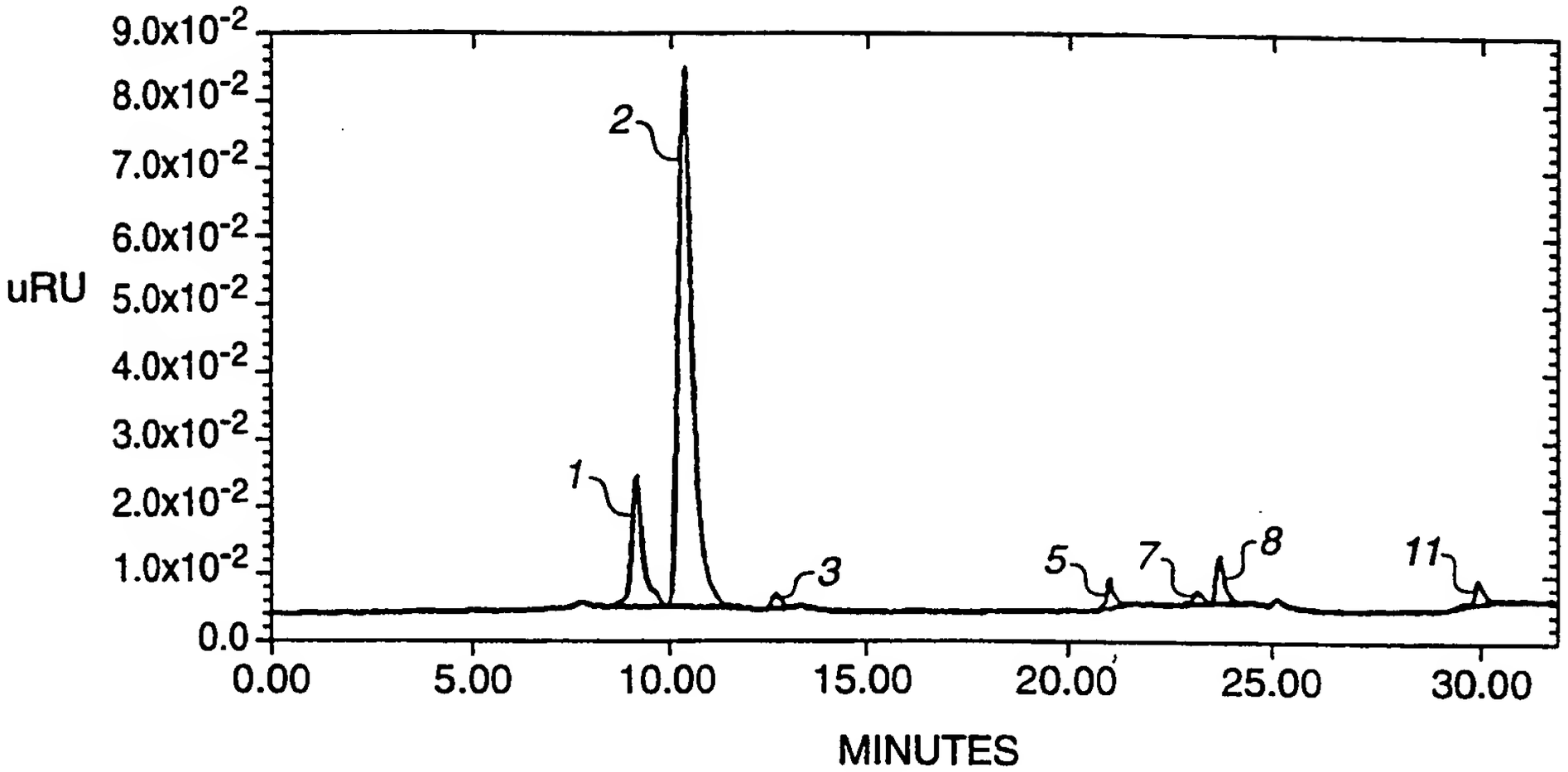

FIG._12A

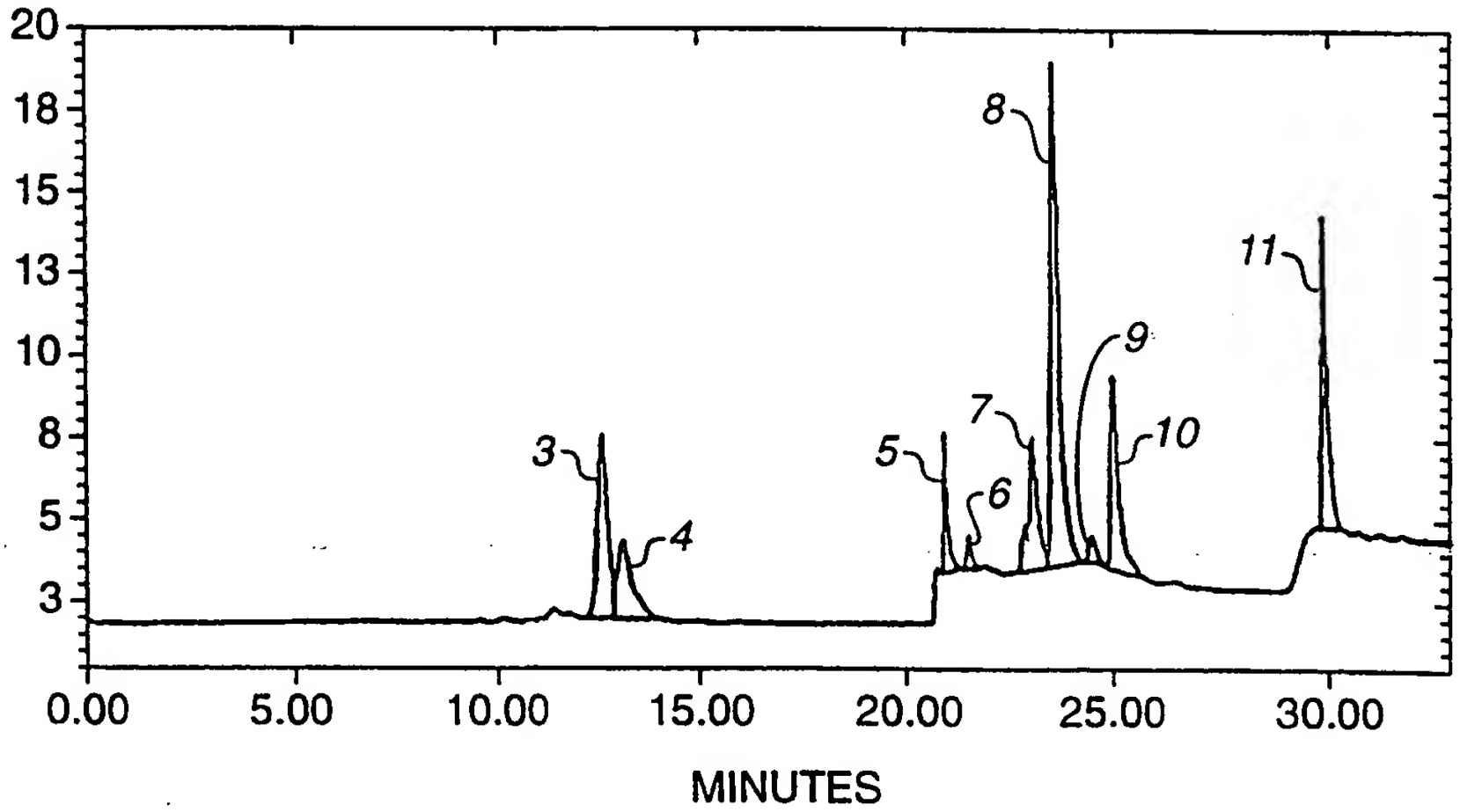

FIG._12B

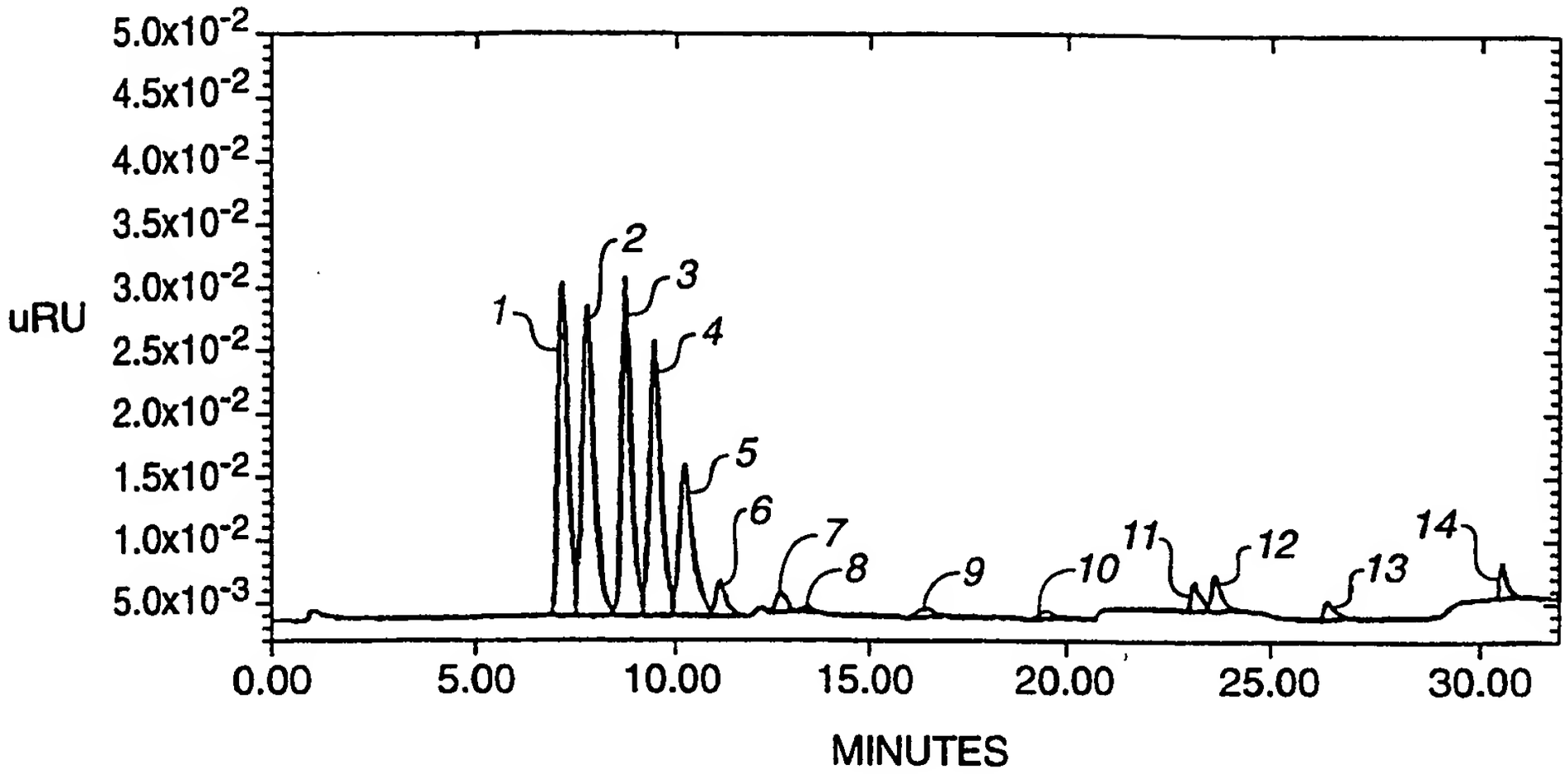

FIG._13A

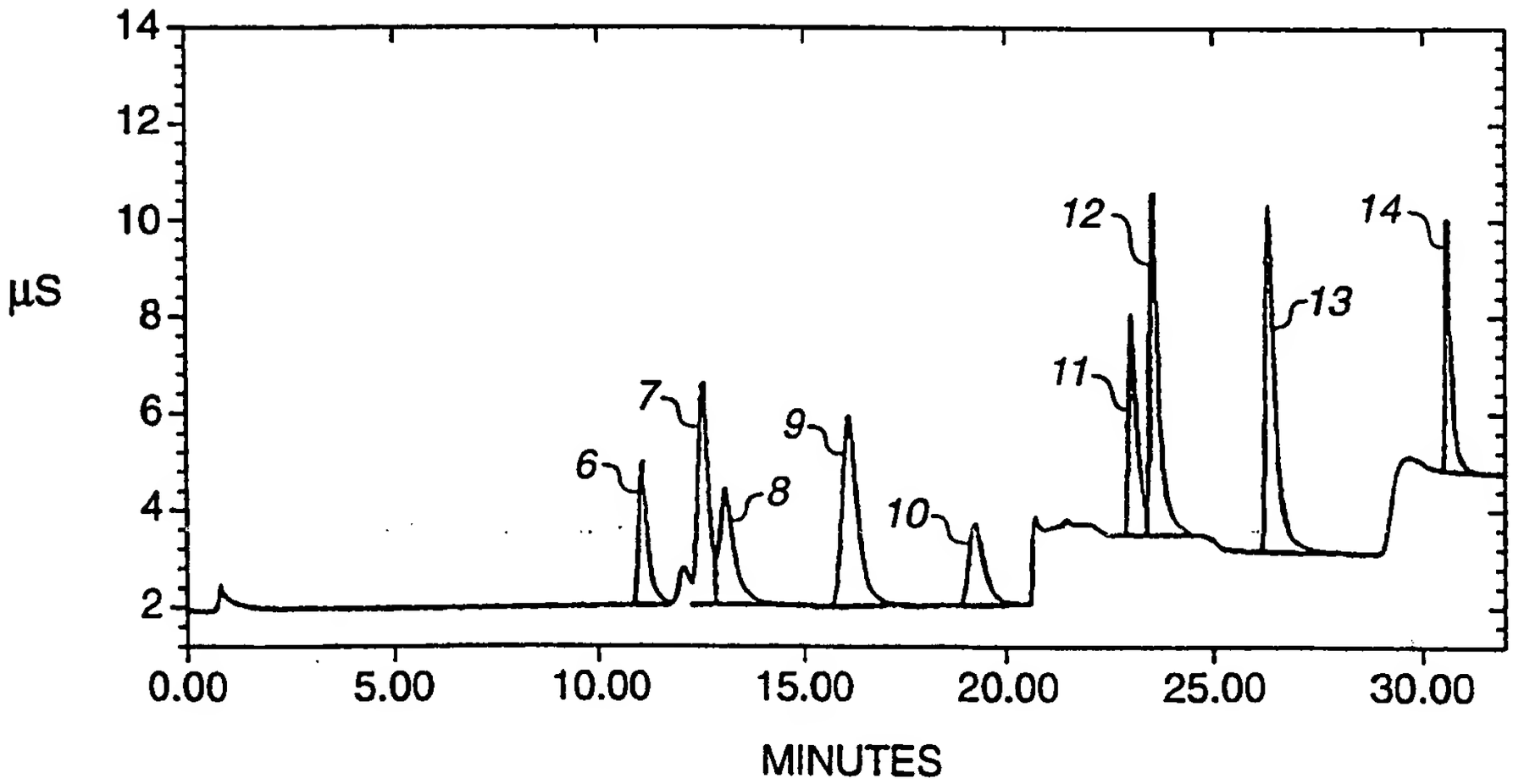

FIG._13B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18647

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B01D 15/00, 15/04, 15/08
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 73/61.52; 210/85, 198.2, 502.1, 635, 656, 660, 661, 679; 422/50, 70; 502/400, 401, 405, 506, 507

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MCFEETERS. R.F. Journal of Agriculture Food Chemistry. 1993. Vol. 41. No. 9. pages 1439-1443.	13, 15-16, 23, 29-30
Y	STEFANSSON. M. et al. Journal of Chromatography A. 1996. Vol. 720. pages 127-136.	1-2, 4, 6-7, 12-14, 19-20, 23-24, 27-30
Y	US 5,084,104 A (HEIKKILA et al) 28 January 1992 (28.01.92), see entire document.	1, 3, 8, 12-13, 19-20, 23, 26, 29-30
Y	US 4,351,909 A (STEVENS) 28 September 1982 (28.09.82), see entire document.	9-11, 20, 22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
19 NOVEMBER 1998	18 DEC 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SUN UK KIM Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18647

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3,920,397 A (SMALL et al) 18 November 1975 (18.11.75), see entire document.	15, 17-19, 31
Y	US 5,324,752 A (BARRETTO et al) 28 June 1994 (28.06.94), see entire document.	9-11, 20, 22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18647

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

73/61.52; 210/85, 198.2, 502.1, 635, 656, 660, 661, 679; 422/50, 70; 502/400, 401, 405, 506, 507